COMPREHENSIVE ASSESSMENT OF A PRE-WORKOUT DIETARY SUPPLEMENT WITH AND WITHOUT SYNEPHRINE

A Dissertation

by

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ABSTRACT

The aim of this study was to examine the effect of acute (Study 1) and chronic (Study 2) ingestion of a pre-workout supplement with (PWS+S) and without *p*-synephrine (PWS) on safety, metabolism, body composition and performance. In Study 1, 25 healthy, recreationally active participants ingested a dextrose flavored placebo (PLA); PWS containing 2 g arginine alphaketoglutarate, 3 g β-alanine, 270 mg caffeine, 2 g creatine nitrate, 15 mg L-Dopa, 300 mg N-acetyl L-tyrosine; or, the PWS with 20 mg *p*-synephrine, interspersed with a 7-d washout, in a randomized, crossover, counter-balanced manner. Participants were tested at time 0 (unsupplemented), and then 30-min and 90-min post ingestion. Data were analyzed by repeated measure MANOVA and reported as mean ± SD or mean change and 95% CI. Both treatment groups reported a greater sense of vigor and energy prior to exercise; PWS+S and PWS scored significantly higher on a Stroop Test. We observed significant differences in RER [PLA (0.89 ± 0.09) vs. PWS (0.92 ± 0.06, $p < 0.02$) and PWS+S (0.85 ± 0.04, $p = 0.006$) and PWS vs. PWS+S ($p < 0.001$)]. In Study 2, 80 resistance trained male were randomized and ingested supplements; PLA, PWS, or PWS+S for 8-wk with resistance training. Testing occurred at baseline, wk 4, and wk 8. Data were analyzed by repeated measure MANOVA and reported as mean ± SD or mean change and 95% CI. We found significant increases in 1RM-Bench Press at wk 4 for PWS and PWS+S, but not for PLA (5.45 kg, 95% CI, -0.82, 11.73). By wk 8 each group demonstrated significant 1RM-BP for PWS, PWS+S and PLA. A similar pattern was noted for 1RM-Leg Press at wk 4 for PWS and PWS+S, but not the PLA (36.50, 95% CI, -0.21, 73.2). By wk 8, all groups increased 1RM-LP. Our data suggest that a PWS

appears safe for acute ingestion and is efficacious regarding indices of cognitive function and exercise performance. We also found that ingesting a PWS during training appears safe, and the inclusion of *p*-synephrine is unnecessary to achieve the observed favorable effects to training adaptation.

DEDICATION

To my parents, Dongman Jung and Jaehee Choi, and my wife, Jae Rhee.

Mom and Dad, I am very grateful to you for all of your love and support, and above all patience and wait. You have always trusted in my decisions and encouraged me to do the best I could. I could not have done this without two of you.

My beloved, thank you for your love. You have always been with me along my side through the entire journey. You have been my best friend and my deepest prayer supporter. I am sure that without your help and prayer, I would not have been able to be here where I am now. We are on the second round of our life, and I will forever be with you.

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NOMENCLATURE

PWS	Pre-workout supplement
PWS+S	Pre-workout supplement with *p*-synephrine
PLA	Placebo
$\dot{V}O_2$	Oxygen consumption or oxygen intake
$\dot{V}CO_2$	Carbon dioxide consumption or carbon dioxide intake
$\dot{V}_E$	Ventilatory equivalent
RER	Respiratory exchange ratio
REE	Resting energy expenditure
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
HR	Heart rate
RHR	Resting heart rate
DXA	Dual x-ray absorptiometry
BMD	Bone mineral density
LM	Lean mass
FM	Fat mass
FFM	Fat free mass
RTP-VAS	Readiness to perform visual analogue scale
1RM	1 repetition maximum
CBC	Complete blood count

RBC	Red blood cell
WBC	White blood cell
PLT	Platelet
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
BUN	Blood urea nitrogen
CRE	Creatinine
CK	Creatine kinase
LDH	Lactate dehydrogenase
LDL-C	Low density lipoprotein cholesterol
HDL-C	High density lipoprotein cholesterol
ECG	Electrocardiogram
BIA	Bioelectrical impedance analysis
TBW	Total body water
ECW	Extracellular water
ICW	Intracellular water
WAT	Wingate 30-sec anaerobic capacity test
CHO	Carbohydrate
FAT	Fat
PRO	Protein
ED	Energy drinks

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CHAPTER I

INTRODUCTION AND RATIONALE

Background

While dietary supplementation of various nutrients during training represents the most common means to enhance exercise performance [1-3], a number of nutritional strategies have been recently purported to affect acute performance by affecting physical, mental, and/or psychological performance [3-6]. For example, dietary supplements containing amino acids, caffeine, creatine, and vitamins have been reported to improve exercise capacity [3, 7, 8], muscle mass and strength [8], muscular endurance [9], and/or subjective feelings of focus and energy [10]. Additionally, the long term ingestion of pre-workout supplements containing L-arginine, β-alanine, caffeine, and creatine have been reported to improve exercise capacity [11], body composition [12], muscle mass and strength [13], and/or subjective feelings of focus and energy [10].

L-arginine is a "semi-essential" amino acid [14] that plays an important role in the synthesis of metabolically active compounds, including other amino acids, muscle protein, and creatine [15]. L-arginine is the substrate of a family of enzymes named nitric oxide synthases (NOSs), and results in the release of nitric oxide (NO) and L-citrulline [16]. NO is critically important for hemodynamics by controlling coronary [17], pulmonary [18], adrenal [19], and systemic vascular tone [20]. Carnosine, a cytoplasmic dipeptide (β-alanyl-L-histidine), is found in highest concentration in the skeletal muscle, whereby the chronic supplementation of β-alanine elevates muscle carnosine content by 40-80% [21] and attenuates the fall in blood pH during high-intensity exercise [22]. This ergogenic

mechanism may lead to an enhanced muscle buffering capacity by limiting the accumulation of hydrogen ions (H^+) [23], and therefore it results in performance enhancement in high intensity anaerobic exercise [24] and delays fatigue [25]. Unlike the hemodynamic and hematologic change observed with L-arginine and β-alanine, caffeine is a central nervous system (CNS) stimulant, due to the binding of caffeine to benzodiazepine receptors [26]. Caffeine has demonstrated multiple beneficial effects for anaerobic power [27], high intensity exercise [28], and endurance performance [29]. Creatine is a naturally occurring compound [30] that is a member of the guanidine phosphagen family [31], and dietary nitrate is abundant in green leafy vegetables and beetroot [32].

The interest in pre-exercise ergogenic supplements following the ban of ephedra by the Food and Drug Administration (FDA) in 2004 has given rise to a number of pre-workout supplement formulae combining various ingredients in an effort to enhance exercise and cognitive performance [33]. On such ingredient considered to be a metabolic stimulant and appetite suppressant is *Citrus aurantium* [34]. Historically, the *Citrus aurantium* has been mainly used for a variety for clinical applications, including indigestion, diarrhea and dysentery, constipation, and as an expectorant in traditional Asian medicine [35]. *p*-synephrine is an analog of ephedra found in the peel of *Citrus aurantium* or its extract [36] and has been used in weight loss dietary supplements due to its thermogenic effects [37]. In a review of the safety and efficacy characteristics of *p*-synephrine, Haaz et al. [38] reported the *p*-synephrine alkaloid potentially increased energy expenditure and decreased food intake, as well as improving gastric motility and

appears to be safe with no serious adverse effects being directly attributable the compound itself [39]. Theoretically, adding *p*-synephrine to PWS formulations may therefore promote thermogenesis, increase perceptions of readiness to perform and/or cognitive performance, and possibly affect exercise capacity.

Statement of the Problem

Is a multi-ingredient pre-workout dietary supplement with *p*-synephrine (PWS+S) safe, more efficacious to enhance performance than placebo (PLA) or PWS only (PWS) during acute and chronic ingestion?

Purpose of the Study

The primary aim of this study is to perform a two-phase series of clinical trials examining the (1) acute and (2) chronic response to a PWS formulated with (PWS+S) and without *p*-synephrine (PWS). The secondary aim of this study is to determine whether the form with *p*-synephrine is safe. The tertiary aim of this study is to examine which form of PWS is more efficacious to improve cognitive and exercise performance.

General Study Overview

There were two experiments to perform the study. For Study 1, 25 healthy and recreationally active volunteers were recruited. The volunteers signed a study consent form, were underwent medical examination and familiarized to the study protocol. The volunteer who did not consent or was not medically cleared were withdrawn. At Day 1, the volunteers were randomized to one of double-labeled supplement groups; PLA, PWS, or PWS+S, and then they were provided with alternate supplement in counter-balanced manner after 7-day washout period. For Study 2, 80 resistance trained male volunteers

were recruited. In same manner of Study 1, the volunteers participated in the study. After all cleared, the eligible volunteers were asked to record 4-day food-log and invited to the baseline testing session (Day 1). After baseline testing, the volunteers were randomized to one of supplement groups, and then followed 8 week supplement and training protocol. The volunteers were asked to check-in the Exercise and Sport Nutrition Lab at Texas A&M University to perform a series of testing the same as baseline testing at week 4 and week 8. During Study 2, the volunteers were instructed to take 12 grams of supplement 15-30 minutes prior to exercise and maintain their current diet throughout the study.

Hypotheses

The central hypotheses are:

Study 1

$H_0$1: There will be significant differences of $\dot{V}O_2$, $\dot{V}CO_2$, and RER among groups for 30 minutes following supplementation.

$H_0$2: There will be significant differences of cognitive function with Stroop Word-Color test among groups prior to, during, and post-supplementation.

$H_0$3: There will be significant differences of readiness to perform among groups prior to, during, and post-supplementation.

$H_0$4: There will be significant differences of upper body strength with bench press test among groups after supplementation.

$H_0$5: There will be significant differences of lower body strength with leg press test among groups after supplementation.

$H_0$6: There will be significant differences of anaerobic capacity with WAT among groups after supplementation.

$H_0$7: There will be no significant differences of resting heart rate and blood pressure among groups prior to, during, and post-supplementation.

$H_0$8: There will be no significant differences of blood metabolic markers; total cholesterol, HDL-C, LDL-C, and glucose, among groups prior to and 2-hr post-supplementation.

$H_0$9: There will be no significant differences of muscle enzymes; LDH and CK, among groups prior to and 2-hr post-supplementation.

$H_0$10: There will be no significant differences of kidney enzymes; CRE and BUN, among groups prior to and 2-hr post-supplementation.

$H_0$11: There will be no significant differences of liver enzymes; ALP, ALT, and AST, among groups prior to and 2-hr post-supplementation.

Study 2

$H_0$12: There will be no significant differences of training volume of upper and lower body among groups over 8 weeks following supplementation.

$H_0$13: There will be no significant differences of consumption of macronutrient; CHO, FAT, and PRO, among groups over 8 weeks following supplementation.

$H_0$14: There will be significant differences of fat mass with DXA body scan among groups over 8 weeks following supplementation.

$H_0$15: There will be significant differences of cognitive function with Stroop Word-Color test among groups over 8 weeks following supplementation.

$H_0$16: There will be significant differences of readiness to perform among groups over 8 weeks following supplementation.

$H_0$17: There will be significant differences of upper body strength with bench press test among groups over 8 weeks following supplementation.

$H_0$18: There will be significant differences of lower body strength with leg press test among groups over 8 weeks following supplementation.

$H_0$19: There will be significant differences of anaerobic capacity with WAT among groups over 8 weeks following supplementation.

$H_0$20: There will be no significant differences of blood metabolic markers; total cholesterol, HDL-C, LDL-C, and glucose, among groups over 8 weeks following supplementation.

$H_0$21: There will be no significant differences of muscle enzymes; LDH and CK, among groups over 8 weeks following supplementation.

$H_0$22: There will be no significant differences of kidney enzymes; CRE and BUN, among groups over 8 weeks following supplementation.

$H_0$23: There will be no significant differences of liver enzymes; ALP, ALT, and AST, among groups over 8 weeks following supplementation.

Delimitations

Study 1

1. The volunteers were asked to not exercise for 48 hours nor eat or drink calorie containing foods or drinks 10-12 hours before each testing session.
2. The volunteers were instructed to refrain from ingesting caffeine and over the counter medication with known stimulant use for 48 hours.
3. The volunteers were scanned to measure the body composition with DXA.
4. The volunteers were asked to donate blood after 12-h fasting on testing day.
5. The volunteers were asked to perform their maximal ability with verbal encouragement on all exercise and testing measures.
6. There were not any performance tests as part of the study protocol.
7. The volunteers were limited to healthy and recreationally active individuals between the ages of 18 to 40.
8. The volunteers were asked to take a break no more than 5 minutes during testing session.

Study 2

9. The volunteers were asked to not exercise for 48 hours nor eat or drink calorie containing foods or drinks 10-12 hours before each testing session.
10. The volunteers were instructed to refrain from ingesting caffeine and over the counter medication with known stimulant use for 48 hours.
11. The volunteers were scanned to measure the body composition with DXA.
12. The volunteers were asked to donate blood after 12-h fasting on testing day.

13. The volunteers were asked to perform their maximal ability with verbal encouragement on all exercise and testing measures.
14. The volunteers were advised to maintain a consistent workout regimen throughout the study.
15. The volunteers were limited to healthy and resistance trained (6-month history of resistance training) male between the ages of 18 to 40.
16. The volunteers were advised to complete a 4-day dietary record (3 weekdays and 1 weekend) a week before baseline testing and 1st day of supplementation, and then a week before the testing day at week 4 and week 8.
17. The volunteers were advised to complete an 8-week resistance training record (2-day upper body and 2-day lower body) throughout the study.
18. The volunteers were instructed to intake the one packet (12 gram of supplement) 15-30 minutes before exercise on training day, and the one packet after breakfast on non-training day, and then were asked to take the empty packet back.
19. The volunteers were refrained from drink other than water during testing session and then provided glucose-electrolyte sport drinks after completing all testing.
20. The volunteers were asked to donate muscle sample from the vastus lateralis at pre- and post-supplementation (week 8).
21. The volunteers were instructed to report any side-effects in a weekly questionnaire.

Limitations

1. The volunteers were individuals from the Texas A&M University community, Bryan/College Station area, and surrounding fitness facilities that respond to recruitment fliers and emails; therefore, the selection process does not truly random.
2. While all effort was made to conduct testing sessions at the same approximate time to account for diurnal variations, there might be some variations in testing times and dietary intake.
3. While the volunteers were required to maintain a consistent training program with supplementation and keep recording daily training-log, there might be some variations in the volume and intensity of individual's workout.
4. While the volunteers were encouraged to make the best effort to perform exercise testing, their intrinsic motivations and effort during the exercise bout and subsequent performance testing might not be maximized at each testing session.
5. While the volunteers were instructed to follow the supplementation protocol, there might be some variations in an actual individual's regimen.
6. While all experiment equipment was calibrated according to manufacturer guidelines and all samples were run under the same condition, there might be some innate limitations of the laboratory equipment that was used for data collection and analysis.

7. While the volunteers were interviewed and recruited by confirmed inclusion/exclusion criteria, there were innate limitations and variability in selection of volunteers.

Assumptions

1. The volunteers followed the overall study protocol and guideline that was explained and consented during familiarization session.
2. The volunteers adhered to 48-hour non-exercise and 12-hour fasting prior to each testing session.
3. The volunteers adhered to the 8-week supplementation protocol that would be explained and consented.
4. The volunteers recorded their 4-day diet-log and 4-day training-log, accurately and honestly.
5. The volunteers answered the entrance questionnaires and side-effect weekly report in a regular basis, accurately and honestly.
6. The trained research staff calibrated and ran all laboratory equipment to maintain consistent.
7. The study coordinator randomized the volunteers and allocated to each supplement group evenly.
8. The volunteers and research staff remained blinded to the assigned supplement throughout the study.

CHAPTER II

REVIEW OF LITERATURE*

Pre-Workout Supplement

While carbohydrate and/or protein supplements were a majority of dietary supplements for performance enhancement in past years [1, 40], a variety of dietary strategies were developed to enhance physical, mental, and even psychological performance in recent decade [4-6, 41-47]. Besides, in recent studies, it was reported energy drinks had similar effects to workout dietary supplements. One of the findings from these literatures is to provide appropriate dietary supplements as an ergogenic aid prior to exercise. Additionally, with resistance training, dietary supplements containing amino acids, caffeine, creatine, and vitamin have been reported to improve exercise capacity [11, 48-52], body composition [12, 53], muscle mass and strength [8, 13, 54, 55], muscular endurance [56, 57], and/or subjective feelings of focus and energy [10, 58]. In Asia, many herbal supplements from vegetable and fruit also have been widely used as ergogenic aids, natural medication, or weight loss treatments. Among them, beetroot as a source of dietary nitrate are used to enhance oxygen uptake [59, 60], elevate metabolic rate [61], and improve exercise performance [62-66]. Velvet bean, *Mucuna pruriens*, extract is an established herbal drug used for the management of male infertility, nervous disorders, and also as an aphrodisiac [67]. In addition, bitter orange, *Citrus aurantium*, is used in herbal medicine as a stimulant and appetite suppressant [34], and it was developed for weight management supplement.

* Creatine section in this chapter is reprinted with permission from Kreider RB and YP Jung. Creatine Supplementation in Exercise, Sport, and Medicine. *Journal of Exercise Nutrition and Biochemistry*. 15(2): 53-69, 2011. DOI: 10.5717/jenb.2011.15.1.053.

In addition to PWS, energy drinks (ED) have been used as an ergogenic aid among active population. A main ingredient of ED is caffeine, and typically caffeinated energy drinks contain from 25 to 250 mg of caffeine in various forms [68]. Shearer et al. [68] reported caffeinated ED increased 1.64 minute (3.6%) of performance time by comparing 24 studies. Administration of 1.3 mg/kg of caffeine resulted in an increased in the running distance in team sports such as rugby or soccer [69], in particular. In International Society of Sports Nutrition (ISSN) position stand regarding energy drink [33], it was reported that consuming in low-to-moderate dosages (3-6 mg/kg) is effective for enhancing sport performance in trained athletes, while there was no further enhancement in performance with higher dosages (>9 mg/kg). Prior to muscle endurance exercise, ingestion of ED (2 mg/kg caffeine) increased approximately 6% more repetitions of failure to 3 sets 70% 1RM [70]. Other than caffeine, guarana, natural source of caffeine, citrulline malates, and synephrine were suggested to affect performance, as well. Further these stimulants mainly have benefits to reaction time, concentration, alertness, and subjective feelings of energy which are important in competitive activity such as hitting a baseball or returning a serve in tennis [33].

Arginine α-ketoglutarate (AAKG)

L-arginine is a semi-essential amino acid [14] that plays an important role in the synthesis of metabolically active compounds, including other amino acids, muscle protein, and creatine [15, 30]. L-arginine is a natural constituent of dietary proteins and classified as a glucogenic amino acid because it can be metabolized into α-ketoglutarate (AKG) entering the citric acid cycle [14]. It was proposed that tricarboxylic acid cycle

intermediates (TCAIs) intake might increase physical performance due to the fact that theoretically TCAIs are limiting factors of TCA cycle, and its supplementation will help to lift these limitation [71]. In addition, administration of AKG with calcium carbonate was reported to effectively improve amino acid metabolism in hemodialysis patients as it decreases hyperphosphatemia [72]. L-arginine as the substrate of a family of enzymes named nitric oxide synthases (NOSs), results in the release of nitric oxide (NO) and L-citrulline [16]. Nitric oxide produced by this pathway is critically important for hemodynamics by controlling coronary [17, 73, 74], pulmonary [18], adrenal [19, 75], and systemic vascular tone [20, 76].

For the theoretical background, L-arginine has been introduced first as a "Cardio-enhancing Supplement" by Fried et al. in 1999 [77]. Maxwell et al. [78] showed administration of L-arginine restored exercise-induced endothelium-derived nitric oxide synthesis and normalized aerobic capacity in hypercholesterolemic mice. In clinical trials, Nagaya et al. [79] suggested L-arginine supplementation might have beneficial effects on hemodynamics and exercise capacity in patients with precapillary pulmonary hypertension. Further, Campbell et al. [14] observed an acute ingestion of arginine-AKG (4 gram) showed significant difference of plasma arginine levels in blood, and 12 grams supplementation (3 times of 4 g/day) with resistance training for 8 weeks had positive effects of 1 RM bench press and Wingate peak power performance in trained adult men. Chen et al. [80] showed an ergogenic benefit of arginine and antioxidant-containing supplement on anaerobic threshold in elderly cyclists, and similarly Yavuz et al. [81] showed arginine alone supplement group (1.5 gram per 10 kg) for elite male wrestlers had

longer time to exhaustion of cycle ergometer compared with placebo group. Among the studies regarding multi ingredients dietary supplements, Camic et al. [82] found arginine-based supplement (3 gram of arginine) to healthy college-aged men for 4 weeks increased gas exchange threshold and power output, and Zak et al. [15] also supported arginine-based supplement (3 gram of arginine) might be used to delay the onset of neuromuscular fatigue and improve the ventilatory threshold in untrained individuals.

β-alanine

Carnosine, a cytoplasmic dipeptide (β-alanyl-$_{L}$-histidine), is found in highest concentration in the skeletal muscle (5-10 mM) and other excitable tissues such as nervous tissue [83]. Due to its acts as a physicochemical buffer in myocytes, it is present mostly higher concentrations in glycolytic than in oxidative muscle fibers [83]. Derave et al. [83] concluded increased muscle carnosine levels attenuated fatigue in repeated bouts of exhaustive dynamic contraction in trained sprinters, and Baguet et al. [22] also suggested that exercise performance improved by high carnosine was because of increase in the non-bicarbonate muscle buffering capacity, increase in the sensitivity of calcium release channels and/or the calcium sensitivity of the contractile apparatus, decrease in the reactive oxygen species (ROS) accumulation, and vasodilation.

Several studies reported the chronic supplementation for 4 week to 8 week of β-alanine elevates muscle carnosine content by 40-80% [21, 83, 84]. del Favero et al. [85] reported 3.2 gram of β-alanine supplement for 12 week was effective in increasing 85.4% of the muscle carnosine content of the gastrocnemius muscle in healthy elderly subjects. For 4 weeks, 6.4 g/day of β-alanine supplementation showed also a significant increase in

the muscle carnosine content of all fiber phentotype, with no significant difference between types [86]. Harris et al. [87] found the supplementation of 3.2 g/day and 6.4 g/day of β-alanine, and total 364 gram of $_{L}$-carnosine for 4 weeks resulted in significant increases in muscle carnosine estimated at 42.1%, 64.2% and 65.8%, respectively. Danaher et al. [88] reported a coingestion of β-alanine and sodium bicarbonate ($NaHCO_3$) elevated buffering potential by increasing muscle carnosine and blood bicarbonate levels, respectively.

Aside from a change of muscle carnosine level, β-alanine supplementation attenuates the fall in blood pH during high-intensity exercise [22], and this ergogenic mechanism may lead to an enhanced muscle buffering capacity by limiting the accumulation of hydrogen ions (H^+) [23]. In healthy elderly study of del Favero et al. [85], it was observed that 36.5% of time-to-exhaustion in the constant-load submaximal test. Like the elderly, college wrestlers and football players ingesting 4 gram of β-alanine for 8 weeks also showed improved performance, decrease in 300 shuttle time by 1.1 seconds and increase in 90° flexed-arm hang [89]. In football players with 4.5 gram of β-alanine supplement for 30 days during training camp, a trend was observed for a lower fatigue rate for supplement group compared with placebo group during the 60 second Wingate anaerobic power test [25]. High intensity interval training (HIIT) with β-alanine supplement (1.5g/day) for six weeks led to improve maximal oxygen consumption rate (VO_{2peak}), cycle ergometer workload at the ventilatory threshold (VT_w), and even body composition; decrease in fat mass and increase in fat free mass [24]. Therefore β-alanine

supplement results in performance enhancement in high intensity anaerobic exercise [24, 85, 89] and delays fatigue [25].

Caffeine

Unlike the hemodynamic change observed with L-arginine and β-alanine, caffeine ($C_8H_{10}N_4O_2$) was considered to be a central nervous system (CNS) stimulant. Due to the characteristics of high lipid solubility, caffeine readily crosses the blood-brain barrier both by diffusion and by a saturable transport system [90]. In this paper, Nehlig et al. [90] also proposed three main mechanisms of action of caffeine, intracellular mobilization of calcium, inhibition of phosphodiesterases, and antagonism at the level of adenosine receptors. With these mechanisms, Nehlig and Debry suggested caffeine increases production of plasma catecholamines that allow the body to adapt to the stress increased by physical activities and increases muscle contractility that improve time to exhaustion, physical performance, and endurance during prolonged activity of submaximal intensity [91].

Due to these facts, caffeine is one of the most widely used ergogenic aids, and various forms of caffeine supplements have become more available over the commercial market in recent years [92-100]. Furthermore, such ergogenic effects of caffeine as metabolic, hormonal, physiologic and cognitive function have been shown in various ranges of studies [97, 101-107]. Among these studies, Ahrens et al. [101] found 6 mg/kg of caffeine ingestion improved oxygen uptake (VO_2), rate of energy expenditure, percentage of maximal oxygen uptake ($\%VO_2$) compared with placebo in women. Additionally, Anselme et al. [107] found caffeine increased blood lactate concentration.

Further, Birnbaum et al. [102] revealed 7 mg/kg of caffeine prior to submaximal running provided a modest ergogenic effect via improved respiratory efficiency and a psychological lift of cross-country runners. While 5 mg/kg of caffeine did not showed a significant change of maximal anaerobic capacity and anaerobic power, it induced significant increases in both catecholamine and blood lactate [27]. The findings of Hogervorst et al. [97] supported caffeine improved cognitive performance by showing better cognitive performance of caffeine group than other groups.

In the studies regarding caffeine supplement and performance, caffeine has demonstrated multiple beneficial effects for anaerobic power [27, 108], high intensity exercise [28], aerobic endurance [29, 96, 98, 109], strength [93], team sport performance [110, 111], and cognitive performance [97, 104, 105]. Eudy et al. [112] reported an amount of 100-500 mg provided beneficial effects such as increased alertness, stimulation, and euphoric effects. In another recent review, Glade [113] concluded the consumption of moderate amounts of caffeine had beneficial effects of mental energy, cognitive function, and neuromuscular coordination as well as physical performance and endurance enhancement. Koppelstaetter et al. [114] showed 100mg of caffeine intake modulated the functional magnetic resonance imaging (fMRI) signal during working memory processes in brain regions that have been associated with attentional and executive functions. Interestingly, a study of caffeine ingestion effect on mood, concentration, and arousal state during a university lecture revealed 100 mg of caffeinated supplement leads to enhanced perceptual feeling of behavior and mood state during a 75-min lecture [115]. In motor coordination study for Navy SEALs [116], 62 male trainees with taking 200 or 300 mg of

caffeine were enabled to sight the target and pull the trigger faster without compromising shooting accuracy during periods of sleep deprivation combined with other stressors.

Creatine

Creatine is a naturally occurring compound that is a member of the guanidine phosphagen family [31]. Creatine is primarily found in the skeletal muscle (~95 %) with small amounts found in the brain and testes (~5 %). About two thirds of creatine in the muscle is stored as phosphocreatine (PCr) while the remaining amount of creatine is stored as free creatine. The total creatine pool (PCr + free creatine) in the muscle averages about 120 grams for a 70 kg individual [117]. However, the upper limit of creatine storage appears to be about 160 grams of creatine in most individuals [117-119]. About 1-2 % of the creatine in the muscle is degraded into creatinine and excreted in the urine per day. Therefore, the body needs to replenish about 1-3 grams of creatine per day to maintain creatine stores. About half of the daily need for creatine is obtained from the diet. For example, there is about 1-2 grams of creatine in a pound of uncooked beef and salmon [120]. The remaining amount of creatine is synthesized from arginine, glycine, and methionine [121]. Vegetarians have been reported to have muscle creatine stores in the 90-110 gram range [122]. Additionally, some people have been found to have creatine synthesis deficiencies and therefore must depend on dietary creatine intake in order to maintain normal muscle and brain concentrations of creatine and phosphocreatine [123].

The primary metabolic role of creatine (Cr) is to combine with a phosphoryl group (Pi) to form PCr through the enzymatic reaction of creatine kinase (CK). Wallimann and colleagues [124-127] suggested that the pleiotropic effects of Cr are mostly related to the functions of CK and PCr (i.e., CK/PCr system). As adenosine triphosphate (ATP) is degraded into adenosine diphosphate (ADP) and Pi to provide free energy for metabolic activity, the free energy released from the hydrolysis of PCr into Cr + Pi can be used as a buffer to resynthesize ATP. This helps maintain ATP availability particularly during maximal effort anaerobic sprint-type exercise. The CK/PCr system also plays an important role in shuttling intracellular energy from the mitochondria into the cytosol. The CK/PCr energy shuttle connects sites of ATP production (glycolysis and mitochondrial oxidative phosphorylation) with subcellular sites of ATP utilization (ATPases) [125]. In this regard, creatine enters the cytosol through a creatine transporter (CRT). In the cytosol, creatine and associated cytosolic and glycolytic CK isoforms help maintain glycolytic ATP levels, the cytosolic ATP/ADP ratio, and cytosolic ATP-consumption [125]. Additionally, creatine diffuses into the mitochondria and couples with ATP produced from oxidative phosphorylation and the adenine nucleotide translocator (ANT) via mitochondrial CK. ATP and PCr can then diffuse back into the cytosol and help buffer energy needs. This coupling also reduces formation of reactive oxygen species (ROS) and can therefore act as a direct and/or indirect anti-oxidant. The CK/PCr energy shuttle thereby connects sites of ATP production (glycolysis and mitochondrial oxidative phosphorylation) with subcellular sites of ATP utilization (ATPases) in order to fuel energy metabolism [125]. In this way, the CK/PCr system thereby serves as an important regulator of metabolism

which may help explain the ergogenic and potential health benefits of creatine supplementation [31, 124, 128].

Short-term creatine supplementation has been reported to improve maximal power/strength (5-15%), work performed during sets of maximal effort muscle contractions (5-15%), single-effort sprint performance (1-5%), and work performed during repetitive sprint performance (5-15%) [129]. Long-term creatine supplementation appears to enhance the quality of training generally leading to 5 to 15% greater gains in strength and performance [129]. Additionally, most studies indicate that creatine supplementation increases body mass by about 1 to 2 kg in the first week of loading [129]. Although the initial weight gain has been suggested to be related to fluid retention, subjects taking creatine typically gain about twice as much body mass and/or fat free mass during training than subjects taking a placebo (e.g., an extra 1-2 kg of muscle mass during 4 to 12 week of training). No study has reported that creatine supplementation significantly impairs exercise capacity.

Mucuna pruriens (Velvet Bean) Extract

Mucuna pruriens or Velvet bean is a tropical legume widespread throughout Africa and Asia, and it is widely naturalized and cultivated to use a rich source of macronutrient and microelements [130]. *Mucuna pruriens* has been used as a natural herb drug in the clinical treatment of the Parkinson's disease by many countries in Asia. India having a long history of many herbal drugs officially recognizes an usage of herbal drugs is an alternative medicine for anti-diabetes, anti-aging, anti-cancer, as well as anti-parkinsonism [131]. Parkinson's disease, a neurodegenerative disease, is defined by low

levels in the brain of the neurotransmitters dopamine [132]. *Mucuna pruriens* seeds are a good source of the non-protein amino acid $_{L}$-3,4-dihydroxyphenylalanine ($_{L}$-Dopa), a precursor of dopamine [130]. Due to its solubility, dopamine itself cannot cross blood brain barrier (BBB), formed by endothelial cells with the presence of tight junctions. Since dopamine itself is unstable, it should be formed within the brain by conversion of its precursor $_{L}$-Dopa to transit through BBB [132].

In a double blind and randomized control study, it was found that *Mucuna pruriens* possessed advantages over conventional $_{L}$-Dopa preparations in the long term management of Parkinson disease [133]. Daily feeding *ad lib* of three different dosages of 2.5, 5.0, or 10.0 g/kg/day with rat chow to Sprague-Dawley rats had a significant effect on dopamine content in the cortex with no significant effect on levodopa, norepinephrine or dopamine, serotonin [134]. Suresh et al. [135] showed 60 days supplementation of *Mucuna pruriens* significantly reduced reactive oxygen species (ROS) and lipid peroxidation (LPO) production and significant increase in both enzymatic and non-enzymatic antioxidant levels in aged rat sperm. Treatment of *Mucuna pruriens* to infertile men also significantly improved Serum T, luteinizing hormone (LH), dopamine, epinephrine, and norepinephrine levels and reduced levels of follicle stimulating hormone (FSH) and prolactin (PRL) [136]. In exercise-trained men, 2250 mg of a blend of *Chlorophytum borivilianum* root and *Mucuna pruriens* increased serum growth hormone (GH) over time at 60 minutes, 80 minutes, and 100 minutes compared to pre-ingestions [137].

N-Acetyl L-Tyrosine (NALT)

N-Acetyl-L-Tyrosine is a more soluble form of L-Tyrosine and is usually modified from normal tyrosine. In the same way of *Mucuna pruriens* extracts, L-Tyrosine is converted to L-Dopa, and the increase in L-Dopa, as a precursor of dopamine, boosts the synthesis of dopamine. The conversion mechanism of tyrosine to dopamine is well-established. Acute administration of an amino acid mixture that selectively lacks both tyrosine and its precursor phenylalanine has been shown to be effective in decreasing availability of tyrosine to the brain through processes of increased protein synthesis and increased competition for transport across the BBB [138]. In this study, Harmer et al. [138] examined the effect of acute tyrosine depletion on dopamine function in healthy volunteers and found they were impaired at spatial recognition memory and spatial working memory following the tyrosine-free drink.

Nitrate

Unlike creatine high in animal protein source, dietary nitrate is abundant in green leafy vegetables and beetroot [32]. A diet high in NO_3^- has been found to have a beneficial impact on several body function and sports performance as an ergogenic aid [65, 66, 139-143]. In particular, the beetroot, an excellent source of NO_3^-, has been used to improve blood pressure as an alternative of antihypertensive drugs [144]. The NO_3^- can be reduced to NO_2^- and in turn to NO. The NO is an important cellular signaling molecule involved in many physiological and pathological processes, and it affects to blood vessel as a powerful vasodilator with a short half-life of a few seconds in the blood.

Recent evidences suggest that beetroot juice supplementation may positively impact the physiological response to exercise [61, 65, 145-147]. It has been reported that

beetroot juice can enhance NO production in the skeletal muscle, thereby increasing blood flow and improving muscle O_2 delivery [63, 148]. These effects are not only for hypertension patients but also for sports athletes. The Australian Institute of Sport recommended that typical dose used in recent studies of sports and exercise performance be ~5-6 mmol or ~300 mg nitrate provided by a single serve of beetroot, consumed ~2-2.5 hours pre-exercise. The ergogenic effects of beetroot juice supplementation have been tested in various sports, and in a 6 days supplement study for cyclists, in particular, beetroot juice group showed a significance of time-trial performance (BR: 953 ± 18 sec vs. PL: 965 ± 18 sec) and power output (BR: 294 ± 12 W vs. PL: 288 ± 12 W) compared with placebo group [66]. In randomized controlled trial study, acute ingestion of beetroot showed the incremental area under the curve (0-6 h after ingestion) for endothelium-independent vasodilation was greater ($p = 0.017$) and lower for diastolic blood pressure ($p = 0.032$) [149].

While the safety of creatine monohydrate as a dietary supplement has been extensively studied in athletes [150-153], muscular dystrophy patient [154, 155], mitochondrial diseases [156], Parkinson disease [157, 158], fibromyalgia patients [159] and confirmed [160], creatine nitrate is a relatively new and novel form of creatine bound to a nitrate molecule [161]. Creatine nitrate has high water solubility compared with creatine monohydrate or buffered creatine. This form of creatine is currently being tested to see its worth as a nutritional supplement [162], particularly in PWS, and additional research is needed to assess the ergogenic value.

Citrus aurantium (Bitter Orange) Extract

p-synephrine, structurally similar to epinephrine [163], is found in a peel of bitter orange (*Citrus aurantium*) or its extract [36]. As a safe alternative to ephedra, it is marketed and widely used in weight loss or weight management. In a review for safety and efficacy of *p*-synephrine, Haaz et al. [38] reported synephrine alkaloid potentially increased energy expenditure and decreased food intake, as well as decreased gastric motility. Stohs et al. [39] summarized in their review that the use of bitter orange extract and *p*-synephrine appears to be exceedingly safe with no serious adverse effects being directly attributable to these ingredients.

In a study for thermic effect of food extracted from *Citrus aurantium* [164], *Citrus aurantium* increased energy metabolism in women and had no effect on blood pressure and pulse rate while epinephrine excretion was increased by 2.4-fold. A study of acute administration of *p*-synephrine with caffeine in young adults showed a dietary supplement with 13 mg of *p*-synephrine and 176 mg of caffeine lead not to increase cardiovascular stress and lead to increase in fat oxidation in certain population [165]. In a 28-d study in rats, Hansen et al. [166] found the increase in heart rate and blood pressure supplemented with caffeine, but no treatment effect on QT interval without caffeine. Additionally, Kaats et al. [167] found no adverse effects at a dose of up to 98 mg/d of *p*-synephrine for 60-d in a double-blind and placebo-controlled study in healthy adults. However, some case studies have reported *p*-synephrine to have adverse effects [168-170]; yet, no research has examined the interaction between dietary supplement ingredients in combination with *p*-synephrine to resistance trained male during acute and chronic ingestion.

Summary

As previously mentioned in PWS and ED, dietary supplements prior to exercise mainly improve alertness and readiness to perform, and it consequentially leads to performance enhancements. Despite some adverse case reports, *Citrus aurantium* extract appear to be effective ergogenic supplement. In addition to lipolytic activity, *p*-synephrine stimulate glucose consumption by stimulating AMPK activity which thought to be important for mitochondrial adaptations to exercise training [171]. Also, ingestion of *p*-synephrine alone showed greater increase in resting metabolic rate than placebo [172]. Because of these reasons, it would be considered to be a potential PWS.

CHAPTER III

METHODS

Study 1: Acute Supplementation

Study 1 was an acute phase study with participants ingesting each respective supplement one time in a randomized, double blind, crossover manner. Primary outcome variables were readiness to perform exercise. Secondary and tertiary outcomes included the blood pressure, heart rate, resting energy expenditure (REE), hematological, strength, and anaerobic power response to supplementation. Each participant was randomly assigned to ingest the supplements described below in double blind and crossover manner. The experiment was repeated after a 7-d washout period observed between each testing session. Table 1 presents an overview of the Study 1.

Each study was performed at the Exercise & Sport Nutrition Laboratory (ESNL) at Texas A&M University after obtaining ethical approval from the universities ethics committee. Herein we provide a concise overview of Study 1 testing procedures followed by a detailed accounting for all testing methodologies.

Participants

Apparently healthy and recreationally active men and women were recruited and participated in this study after obtaining ethical approval from the universities Internal Review Board. Inclusion criteria required that each participant have at least six months of resistance training experience immediately prior to entering the study inclusive of performing bench press and leg press or squat. Participants were excluded if they

Table 1. Study 1 Protocol Overview

Familiarization(T1)	T2	T3	T4
Phone Screening	Fasting Blood Sample	Fasting Blood Sample	Fasting Blood Sample
Familiarization	Cognitive Function Test	Cognitive Function Test	Cognitive Function Test
Physical Exam	Readiness to Perform VAS	Readiness to Perform VAS	Readiness to Perform VAS
Body Weight	12-Lead Resting ECG	12-Lead Resting ECG	12-Lead Resting ECG
DEXA Body Composition 1RM Determination	REE (10-min) and resting HR & BP	REE (10-min) and resting HR & BP	REE (10-min) and resting HR & BP
Anaerobic Sprint Practice Test Practice	Ingest supplement in randomized and counterbalanced manner	Ingest supplement in randomized and counterbalanced manner	Ingest supplement in randomized and counterbalanced manner
Schedule Testing Refrain from exercise, caffeine and use of over-the-counter stimulants for 48 hours prior to each testing session	REE for 30-min with HR & BP determined every 10-min and arrhythmias documented (if any)	REE for 30-min with HR & BP determined every 10-min and arrhythmias documented (if any)	REE for 30-min with HR & BP determined every 10-min and arrhythmias documented (if any)
	Blood Sample	Blood Sample	Blood Sample
	Cognitive Function Test	Cognitive Function Test	Cognitive Function Test
	Readiness to Perform VAS	Readiness to Perform VAS	Readiness to Perform VAS
	Bench Press Warm-Up	Bench Press Warm-Up	Bench Press Warm-Up
	Bench Press (3 sets of 10 reps @ 70% or 1RM with 2-min rest recovery between sets. Total reps to failure on last set)	Bench Press (3 sets of 10 reps @ 70% or 1RM with 2-min rest recovery between sets. Total reps to failure on last set)	Bench Press (3 sets of 10 reps @ 70% or 1RM with 2-min rest recovery between sets. Total reps to failure on last set)
	5-min rest	5-min rest	5-min rest
	Leg Press Warm-up	Leg Press Warm-up	Leg Press Warm-up
	Leg Press (3 sets of 10 reps @ 70% or 1RM with 2-min rest recovery between sets. Total reps to failure on last set)	Leg Press (3 sets of 10 reps @ 70% or 1RM with 2-min rest recovery between sets. Total reps to failure on last set)	Leg Press (3 sets of 10 reps @ 70% or 1RM with 2-min rest recovery between sets. Total reps to failure on last set)
	5-min rest	5-min rest	5-min rest
	Wingate AC Test	Wingate AC Test	Wingate AC Test
	Cognitive Function Test	Cognitive Function Test	Cognitive Function Test
	Readiness to Perform VAS	Readiness to Perform VAS	Readiness to Perform VAS
	Blood Sample	Blood Sample	Blood Sample
	1-week washout	1-week washout	

presented with a history of treatment for metabolic disease, hypertension, thyroid disease, arrhythmias, and/or cardiovascular disease; and/or, were they are currently using any prescription medication. Further exclusion criteria also included an intolerance to caffeine and/or other natural stimulants; pregnant or lactating women; a history of smoking; and, excessive alcohol consumption (>12 drinks/wk). Participants who met study entry criteria were invited to a familiarization session.

Familiarization Session

During familiarization, the details of the study were explained, informed consent was obtained, and medical history information was completed via a general clinical exam to determine eligibility to participate in the study. At this same session, participants were assessed for height and body mass and had dual energy X-ray absorptiometry (DXA) body composition (excluding cranium) determined.

Following these assessments, participants performed a one repetition maximum (1RM) test on the bench press and leg press. All strength testing took place on an isotonic Olympic bench press and hip/leg sled (*Nebula Fitness, Versailles, OH*) using standard procedures [173]. Maximal strength was determined following a standard warm-up consisting of 10 repetitions using 50% of their estimated 1RM, 5 repetitions using 70% of their estimated 1RM, and 1 repetition using 90% of their estimated 1RM. Participants continued increasing weight until their 1RM's were determined. Participants were encouraged to reach 1RM during the familiarization trials. Previous research in our lab on resistance-trained participants has yielded a low day-to-day mean coefficients of variation and high reliability for the bench press and leg press (1.1%, intra-class, r = 0.99). After

then, participants practiced the Wingate anaerobic capacity testing procedures. Those who were cleared were scheduled to begin baseline testing.

Pre- and Post-Supplementation Testing Procedures

Prior to testing, we instructed participants to refrain from exercise, caffeine, and supplements and/or medications containing stimulants for 48-hr prior to testing. Participants presented to the lab after a 12-hr fast and donated ~ 20 ml of blood via venipuncture. Following blood sampling, we administered a series of tests to assess: cognitive function (Stroop Test), Readiness to Perform Visual Analogue Scale (RTP-VAS), resting blood pressure (BP), resting heart rate (HR), and resting energy expenditure (REE) for 10 minutes.

After 10-min pre-supplementation procedures, each participant was randomized in counterbalanced and double-blinded manner to ingest the respective supplements. Participants were monitored BP, HR and REE every 10-min for 30-min post-supplementation. After rest testing, participants performed a 5-min warm up followed by 3 sets of 10 repetitions at 70% of 1RM on the bench press and leg press interspersed by two minutes of rest between sets and 5-min recovery between each exercise testing modality. During the third set, we asked participants to complete as many repetitions as possible. Following a 5-min recovery, participants performed a standard Wingate test to assess anaerobic capacity peak power (PP), mean power (MP) and total work (TW) on a computerized cycle ergometer. Participants then performed a final series of cognitive function and readiness to perform testing and donated a third blood sample. Figure 1 shows the timeline of testing procedures. The experiment was repeated using the alternate

supplement administered in a counterbalanced manner two additional times following 7-day washout after each additional testing session.

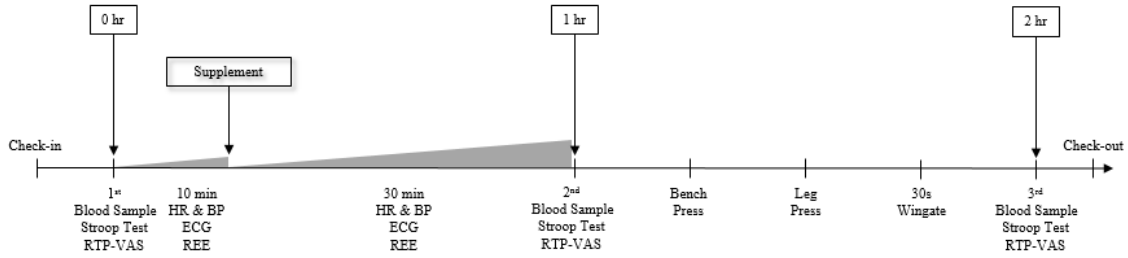


Figure 1. Study 1 Timeline of Measurement Procedures

Supplements

Treatments consisted of a (1) flavored placebo (PLA), (2) a PWS containing arginine alpha-ketoglutarate (2 g), β-alanine (3 g), caffeine (270 mg), creatine nitrate (2 g), N-Acetyl-L-Tyrosine (300 mg), *Mucuna pruriens* extract standardized for 15% L-Dopa (15 mg), Vitamin C as Ascorbic Acid (500 mg), niacin (60 mg), folate as folic acid (50 mg), and Vitamin B_{12} as Methylcobalamin (70 mg) (*Nutrabolt, Bryan, TX*); or, (3) the PWS with *Citrus aurantium* extract standardized for 30% synephrine (20 mg) (PWS+S) (*Nutratech Inc., Caldwell, NJ*). Figure 2 and Figure 3 present the supplement facts of PWS and the product specification of synephrine, respectively.

Supplements were prepared in 12 g of pink powder in A, B, and C coded single foil packets and labeled for double blind administration (A: PWS+S, B: PWS, and C: PLA). Supplements were provided by the supporting, and were counted and pre-packaged by an

individual outside of the research team. The ingredients of the packets were independently verified by VMI Nutrition (*Salt Lake, UT*).

Supplement Facts

Serving Size: 1 scoop (5.7g)
Servings Per Container: 30

	Amount Per Serving	% DV
Calories	5	
Total Carbohydrates	1g	<1%**
Vitamin C	250mg	417%
Calcium	28mg	3%
Niacin (as Niacinamide)	30mg	150%
Folic Acid	250mcg	62%
Vitamin B12	35mcg	588%
Beta Alanine	1500mg	†
Creatine Nitrate	1000mg	†
Arginine AKG	1000mg	†
Explosive Energy Blend	741mg	†

Vitamin C (as Ascorbic Acid), N-Acetyl-L-Tyrosine, Caffeine Anhydrous (135mg), Mucuna pruriens (Standardized for L-Dopa), Bitter orange (Citrus aurantium)(fruit)(30% Synephrine)(Advantra Z®), Niacinamide, Folate (as Folic Acid), Pyridoxal 5-Phosphate, Vitamin B12 (as Methylcobalamin)

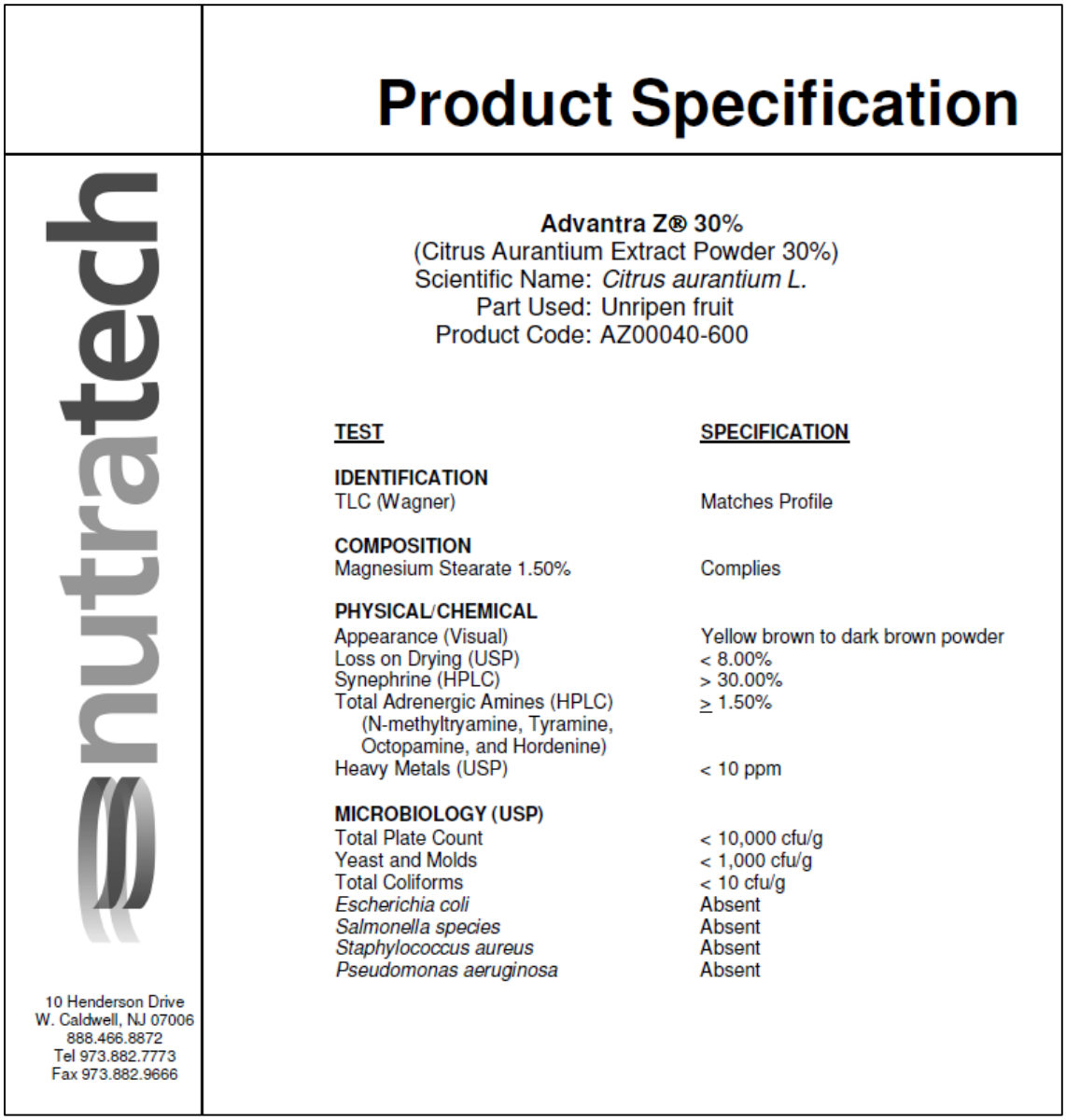
**Percent Daily Values (% DV) are based on a 2,000 calorie diet.
† Daily Value not established.

Other Ingredients: Artificial Flavors, Citric Acid, Malic Acid, Silicon Dioxide, Calcium Silicate, Sucralose, Acesulfame Potassium (Ace-K), FD&C Red Lake #40.
Advantra Z® is a registered trademark of Nutratech Inc. under U.S. patents 6,224,873; 6,316,499; 6,340,481; 6,340,482

Figure 2. Supplement Facts of PWS

Testing Methodologies

Anthropometry: At familiarization session of Study 1, height and weight, and body composition were determined. Body mass and height were determined via a calibrated scale (*Cardinal Detecto Scale Model 8430, Webb City, MO*) and body composition was determine using a Dual Energy X-Ray Absorptiometer (DXA) excluding



Product Specification

Advantra Z® 30%
(Citrus Aurantium Extract Powder 30%)
Scientific Name: *Citrus aurantium L.*
Part Used: Unripen fruit
Product Code: AZ00040-600

TEST	SPECIFICATION
IDENTIFICATION	
TLC (Wagner)	Matches Profile
COMPOSITION	
Magnesium Stearate 1.50%	Complies
PHYSICAL/CHEMICAL	
Appearance (Visual)	Yellow brown to dark brown powder
Loss on Drying (USP)	< 8.00%
Synephrine (HPLC)	> 30.00%
Total Adrenergic Amines (HPLC) (N-methyltryamine, Tyramine, Octopamine, and Hordenine)	≥ 1.50%
Heavy Metals (USP)	< 10 ppm
MICROBIOLOGY (USP)	
Total Plate Count	< 10,000 cfu/g
Yeast and Molds	< 1,000 cfu/g
Total Coliforms	< 10 cfu/g
Escherichia coli	Absent
Salmonella species	Absent
Staphylococcus aureus	Absent
Pseudomonas aeruginosa	Absent

nutratech

10 Henderson Drive
W. Caldwell, NJ 07006
888.466.8872
Tel 973.882.7773
Fax 973.882.9666

Figure 3. Product Specification of Synephrine

cranium (*Discovery W, Hologic Inc., Waltham, MA*). Previous studies indicate DXA to be an accurate and reliable means to assess changes in body composition [174]. For determination of body composition, participants removed all metal objects that are known

to interfere with measurement. Participants were then positioned in the supine position based on manufacture's guideline by a trained technician. DXA measurement was then performed; taking approximately 6-8 minutes. Analysis was immediately performed by a trained technician to determine body composition. Test/retest reliability studies performed on male athletes with DXA yielded mean deviation for total bone mineral content and total fat-free-soft tissue mass of 0.31-0.45%, with a mean intra-class correlations of 0.985 [174].

Blood Chemistry: During testing, total three blood samples were collected at pre-supplement, 60min post-supplement, and 120min post-supplement. All blood samples were analyzed for standard blood chemistries inclusive of alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), creatinine, blood urea nitrogen (BUN), creatine kinase (CK), lactate dehydrogenase (LDH), glucose, and blood lipids (total cholesterol, high density lipoprotein [HDL], low density lipoprotein [LDL], triglycerides [TG]) using a Cobas® c111 (*Roche Diagnostics, Basel, Switzerland*). The Cobas® automated clinical chemistry analyzer was calibrated according to manufacturer guidelines. This analyzer has been known to be highly valid and reliable in previously published reports [175]. The internal quality control for the Cobas® c111 was performed using two levels of control fluids purchased from manufacturer to calibrate acceptable SD and coefficients of variation values for all aforementioned assays. Samples were re-run if the observed values were outside control values and/or clinical norms according to standard procedures. We also assessed a complete blood count with platelet differential on whole blood (hemoglobin, hematocrit, red blood cell counts, MCV, MCH, MCHC, RDW, white blood cell counts, lymphocytes, granulocytes, and mid-range absolute count

(MID) using a Abbott Cell Dyn 1800 (*Abbott Laboratories, Abbott Park, IL, USA*) automated hematology analyzer. The internal quality control for Abbott Cell Dyn 1800 was performed using three levels of control fluids purchased from manufacturer to calibrate acceptable Cv values for all whole blood cell parameters (± 6.27%).

Blood Pressure and Heart Rate Assessment: Resting blood pressure (BP) and resting heart rate (HR) were determined by palpation of the radial artery using standard procedures and aneroid sphygmomanometer. Blood pressure was also assessed using standard auscultatory procedures [176].

Cognitive Function (Stroop Word-Color Test): During testing, cognitive function was tested with Stroop Word-Color test standardized by Golden [177]. The test consists of three pages (Fig. 4). Each page has 100 items, presented in 5 columns of 20 items. Items on the Page 1 (Word) are the color words RED, GREEN, and BLUE in black ink. On the Page 2 (Color) the items are XXX's colored in red, green, or blue ink. Items on the Page 3 (Word-Color) are the words RED, GREEN, and BLUE printed in red, green, or blue ink with the limitation that word and ink could not match. Participants read loudly each page (Word, Color, and Word-Color page in order) for 45 second as fast as they can.

Readiness to Perform Visual Analogue Scale: With same as cognitive function, Readiness to Perform was tested three times and measured by visual analogue scale (VAS) with five subjective feeling (strongly disagree to strongly agree) on 20 cm dotted bar (Fig. 5). The VAS has six questions; (1) "*I slept well last night*", (2) "*I am looking forward to today's workout*", (3) "*I am optimistic about my future performance*", (4) "*I feel vigorous*

Page 1

RED	BLUE	GREEN	RED	BLUE
GREEN	GREEN	RED	BLUE	GREEN
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BLUE	GREEN	RED	GREEN	RED
GREEN	RED	BLUE	RED	BLUE
BLUE	GREEN	GREEN	BLUE	GREEN
GREEN	RED	BLUE	RED	RED
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Page 2

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Page 3

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Figure 4. Stroop Word-Color Test Sheets

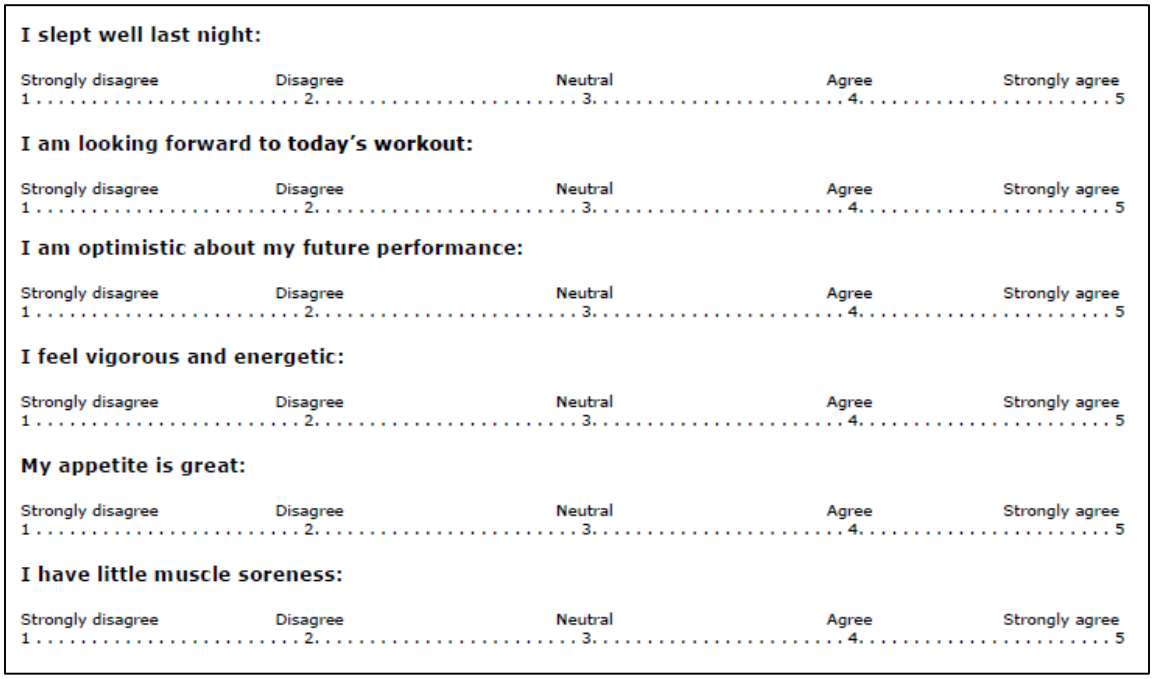

Figure 5. Readiness to Perform Visual Analogue Scale

and energetic", (5) "*My appetite is great*", (6) "I *have little muscle soreness*". Participants circled the number or dotted between numbers that best indicated how they currently felt.

Strength Testing with 1RM: With 1RM determined at familiarization, participants performed 3 sets of bench and leg press test. At the first and second set, participants were asked to lift 10 repetitions at 70% of 1RM on the bench press and leg press interspersed by two minutes of rest between sets and 5-minute recovery between each exercise testing modality. During the third set, we asked participants to complete as many repetitions as possible they can.

Wingate Anaerobic Capacity Testing: Wingate testing was assessed using a Lode Excalibur Sport Ergometer (*Lode BV, Groningen, The Netherlands*) and work rate was set at of 7.5 J/kg/rev. Participants were asked to pedal as fast as possible prior to application

of the workload and sprint at an all-out maximal capacity for 30-sec Wingate test. Test-to-test variability in performing repeated Wingate anaerobic capacity tests in our lab have yielded correlation coefficients of r = 0.98±15% for mean power.

Study 2: Chronic Supplementation

Study 2 was an 8-wk study using different participants receiving supplements in a randomized, double blind manner. Primary outcome variables were exercise performance; upper and lower body strength, and anaerobic power. Secondary and tertiary outcomes included the readiness to perform exercise, blood pressure, heart rate, and hematological response to supplementation. Table 2 presents an overview of the Study 2.

Each study was performed at the Exercise & Sport Nutrition Laboratory (ESNL) at Texas A&M University after obtaining ethical approval from the universities ethics committee. Herein we provide a concise overview of Study 2 testing procedures followed by a detailed accounting for all testing methodologies.

Participants

We recruited male participants and administered the study as a randomized, double blind, placebo-controlled trial lasting eight weeks. Inclusion criteria required that each participant have at least six months of resistance training immediately prior to entering the study inclusive of performing bench press and leg press or squat. Participants were excluded if they presented with a history of treatment for metabolic disease, hypertension, thyroid disease, arrhythmias, and/or cardiovascular disease; and/or were they are currently using any prescription medication. Further exclusion criteria also included an intolerance

to caffeine and/or other natural stimulants; a history of smoking; excessive alcohol consumption (>12 drinks/wk).

Familiarization Session

Participants who met study entry criteria were invited to a familiarization session where the details of the study were explained, signed, informed consent was obtained medical history information was completed via a general clinical exam that included an assessment of fasting blood (i.e., to rule out diabetes) to determine eligibility to participate in the study. Those who were cleared were then scheduled to begin baseline testing.

Baseline Testing

Before baseline testing, we instructed participants to refrain from exercise, caffeine, and supplements/medications containing stimulants for 48-h. prior to testing. Participants presented to the lab after a 12-h fast and were required to provide 4-d food-log recorded their consumption of food a week before testing session. Once checked in, participants donated ~ 20 ml of blood via venipuncture, and we administered a series of tests to assess body composition: body weight, body water (BIA), body composition (DXA). After approximately 30-min rest testing, resting blood pressure (BP) and resting heart rate (HR) were tested.

Following those testing, participants had 5-min break. After then cognitive function tests (Stroop Test) and Readiness to Perform Visual Analogue Scale (RTP-VAS) were tested, and they continued to perform bench and leg press, and Wingate 30s test. Figure 6 shows the timeline of testing procedures.

Table 2. Study 2 Protocol Overview

Familiarization(T1)	T2 (week 1)		T3 (week 4)	T4 (week 8)	
Visit 1	Biopsy ~ 1 day prior Visit 2	Performance testing Visit 3	Performance testing Visit 4	Biopsy ~ 1 day prior Visit 5	Performance testing Visit 6
Phone Screening Familiarization Physical Exam Body Weight 1 Repetition Maximum Practice Familiarization Anaerobic Sprint Test Practice Schedule Testing Refrain from exercise, caffeine and use of over-the-counter stimulants for 48-hours prior to each testing session	Muscle Biopsy	4-Day Food Record 48 hour non-exercise 12 hour fast Fasting Blood Sample Body Weight Body Water Body Composition Readiness to Perform Scale & Cognitive Function Test Bench Press Warm-Up Bench Press 1 Repetition Maximum with 2-min rest recovery between sets. 5-min rest Leg Press Warm-up Leg Press 1 Repetition Maximum with 2-min rest recovery between sets. 5-min rest Wingate Anaerobic Sprint Test Administration of supplements Begin training	4-Day Food Record 48 hour non-exercise 12 hour fast Fasting Blood Sample Body Weight Body Water Body Composition Readiness to Perform Scale & Cognitive Function Test Bench Press Warm-Up Bench Press 1 Repetition Maximum with 2-min rest recovery between sets. 5-min rest Leg Press Warm-up Leg Press 1 Repetition Maximum with 2-min rest recovery between sets. 5-min rest Wingate Anaerobic Sprint Test	Muscle Biopsy	4-Day Food Record 48 hour non-exercise 12 hour fast Fasting Blood Sample Body Weight Body Water Body Composition Readiness to Perform Scale & Cognitive Function Test Bench Press Warm-Up Bench Press 1 Repetition Maximum with 2-min rest recovery between sets. 5-min rest Leg Press Warm-up Leg Press 1 Repetition Maximum with 2-min rest recovery between sets. 5-min rest Wingate Anaerobic Sprint Test

Supplementation Protocol

Following baseline testing, participants entering this phase of the trial were matched for age, body mass, fat free mass (FFM), and training history and subsequently randomized to receive a PLA, PWS and PWS+S as described above. Participants were

assigned in a double-blind and counter-balanced manner and asked to ingest 12 g of supplements with 235 ml of water; once daily approximately 15-30 min prior to exercise on training day and approximately same time every day in the morning with breakfast on non-training day.

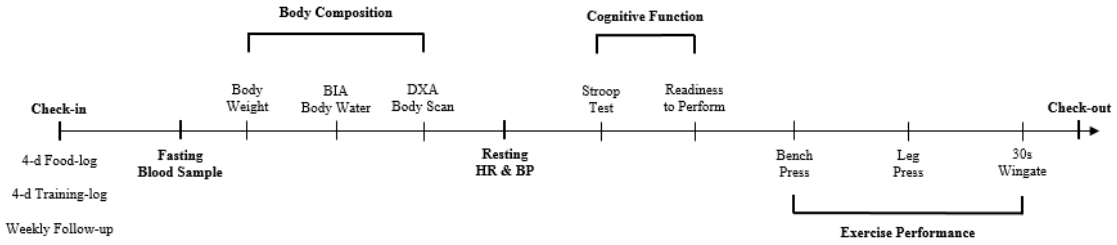

Figure 6. Study 2 Timeline of Measurement Procedures

Training Protocol

Unlike Study 1, all participants were required to follow the same resistance training routine during Study 2. The resistance training routine consisted of exercise 4-d per week split into two upper and two lower body workouts per week for a total of 8-weeks. The 8-wk training protocol was periodized in 3-wk increments consisting of selected exercises for the following muscle groups: chest (two exercises for a total of six sets), back (two exercise for a total of six sets), shoulders (one exercise for a total of three sets), biceps (one exercise for a total of three sets), triceps (one exercise for a total of three sets), abdominals (one exercise for a total of three sets), quadriceps (two exercises for a total of six sets), hamstrings (two exercises for a total of six sets), and calves (one exercise

for three sets). Each exercise consisted of three sets of 10 repetitions (wk 1-3), 8 repetitions (wk 4-6), or 6 repetitions (wk 7-8) performed with as much weight as the participant could perform per set. Training logs were completed and maintained by each participant. The participants recorded the amount of weight lifted during each set on a training log. A training partner or fitness instructor who signed off the session monitored training sessions. Table 3 and Table 4 present the training logs of upper and lower body workout, respectively.

Food Frequency Record

All participants were required to record their food consumption of 4-day a week before at each testing session and instructed to record everything they ate for 3 weekdays and 1 weekend day. Further, participants were asked to precisely record the food item (brand if applicable), preparation method, and total quantity consumed. Table 5 shows the daily food-log table.

Testing Procedures at Week 4 and Week 8

Same as baseline testing procedure, participants were instructed to refrain from exercise, caffeine, and supplements/medications containing stimulants for 48-h prior to testing. Participants presented to the lab after a 12-h fast and were required to provide 4-d food-log, training-log, and weekly follow-up report. Once checked in, participants donated ~ 20 ml of blood via venipuncture, and we administered a series of tests: body weight, body water (BIA), body composition (DXA), resting BP, resting HR, Stroop Test and RTP-VAS test, bench and leg press, and Wingate 30s test. These testing were repeated at week 4 (mid-supplementation) and week 8 (post-supplementation).

Table 3. Study 2 Training-log for Upper Body

MONDAY & THURSDAY UPPER BODY RESISTANCE TRAINING PROGRAM									
Week		1	1	2	2	3	3	4	4
Workout		1	2	3	4	5	6	7	8
Day		Monday	Thursday	Monday	Thursday	Monday	Thursday	Monday	Thursday
Date [M/D/YY]		/ /	/ /	/ /	/ /	/ /	/ /	/ /	/ /
		Reps/Wt	**Reps/Wt**	**Reps/Wt**	**Reps/Wt**	**Reps/Wt**	**Reps/Wt**	**Reps/Wt**	**Reps/Wt**
SECTION I	Bench Press	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Incline Press	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Decline Press	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Machine Press								
	DB Bench Press								
	DB Incline Press								
SECT. II	Chest Flies	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Cable Crossovers	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
		10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
SECT. III	Wide-Grip Lat Pull	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Close-Grip Lat Pull	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
		10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
SECT. IV	Seated Rows	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Bent-Over Row	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	DB Bent-Over Row	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
SECT. V	Shoulder Press	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	DB Shldr Press	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Machine Shoulder Press	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
SECT. VI	Shoulder Shrugs	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
		10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
		10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
SECTION VII	DB Curls	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Preacher Curls	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	DB Preacher Curls	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Barbell Curls								
SECTION VIII	Triceps Pressdown	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Rope Pressdown	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Reverse-Grip Pressdown	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	DB Triceps Ext.								
	Lying Triceps Ext.								
Verification Signature									
****CHOOSE ONLY *ONE* EXERCISE PER BODYPART FOR EACH SECTION****									

Table 4. Study 2 Training-log for Lower Body

TUESDAY & FRIDAY LOWER BODY RESISTANCE TRAINING PROGRAM									
Week		1	1	2	2	3	3	4	4
Workout		1	2	3	4	5	6	7	8
Day		Tuesday	Friday	Tuesday	Friday	Tuesday	Friday	Tuesday	Friday
Date [M/D/YY]		/ /	/ /	/ /	/ /	/ /	/ /	/ /	/ /
		Reps/Wt	**Reps/Wt**	**Reps/Wt**	**Reps/Wt**	**Reps/Wt**	**Reps/Wt**	**Reps/Wt**	**Reps/Wt**
SECTION I	Leg Press	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Barbell Back Squats	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Smith Mach. Squats	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Machine Squats								
SECT. II	Leg Extension	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
		10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
		10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
SECT. III	Machine Back Extension	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Barbell RDL (Romanian Deadlift)	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Barbell Good Mornings	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
SECT. IV	Step-ups (Barbell or DB)	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Lunges (Barbell or DB)	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Split Squats (Barbell or DB)	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
SECT. V	Seated Leg Curl	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Lying/Prone Leg Curl	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
		10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
SECT. VI	Standing Calf Raises	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Seated Calf Raises	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
	Seated Rotary Calf Machine	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	10 x _____	8 x _____	8 x _____
SECT. VII	Abdominal Crunches	25 x body wt	25 x body wt	25 x body wt	25 x body wt	25 x body wt	25 x body wt	25 x body wt	25 x body wt
		25 x body wt	25 x body wt	25 x body wt	25 x body wt	25 x body wt	25 x body wt	25 x body wt	25 x body wt
		25 x body wt	25 x body wt	25 x body wt	25 x body wt	25 x body wt	25 x body wt	25 x body wt	25 x body wt
Verification Signature									
****CHOOSE ONLY *ONE* EXERCISE PER BODYPART FOR EACH SECTION****									

Table 5. Study 2 Daily Food-log Sheet

Instructions:

1) Record everything that you eat for 3 weekdays AND 1 weekend day
2) Precisely record the food item (brand if applicable), preparation method, and TOTAL quantity consumed
3) Break down mixed dishes or recipes by listing their component parts
4) For dairy and meat products, indicate fat level (i.e. low fat, extra lean, 2%, etc.)

FOOD ITEM	PREPARATION METHOD (i.e. baked, fried, grilled, etc.)	QUANTITY							
		gm	mL	cups	T or tsp.	oz.	Pieces	Sm, Med, Lg	Other
MEAL 1:									
MEAL 2:									
MEAL 3:									
MEAL 4:									

Testing Methodologies

Anthropometry: For Study 2, body composition was tested at baseline, week 4 and week 8. Body mass and height, and body composition were determined using an auto-calibrated scale with a Dual Energy X-Ray Absorptiometer (DXA) excluding cranium as the same model and protocol used for Study 1. Test/retest reliability studies performed on male athletes with DXA yielded mean deviation for total bone mineral content and total fat-free-soft tissue mass of 0.31-0.45%, with a mean intra-class correlations of 0.985 [174].

Blood Chemistry: At each testing session, blood sample was collected with 12-h fasting once participants presented. All blood samples were analyzed with the same methods of Study 1. The internal quality control for the Cobas® c111 was performed using two levels of control fluids purchased from manufacturer to calibrate acceptable SD and coefficients of variation values for all aforementioned assays, and the one of Abbott Cell Dyn 1800 was performed using three levels of control fluids purchased from manufacturer to calibrate acceptable Cv values for all whole blood cell parameters (± 6.27%).

Blood Pressure and Heart Rate Assessment: Resting BP and resting HR were determined by palpation of the radial artery using standard procedures and aneroid sphygmomanometer. Blood pressure was also assessed using standard auscultatory procedures [176].

Cognitive Function and Readiness to Perform: Cognitive function and RTP-VAS were tested the same way of Study 1 with Stroop Word-Color test standardized by Golden [177] and with five subjective feeling (strongly disagree to strongly agree) on 20 cm dotted bar, respectively.

1RM Bench and Leg Press Testing: BP-1RM and LP-1RM testing procedure of Study 2 were performed using the same as Study 1 at familiarization. Previously mentioned, research in our lab on resistance-trained participants has yielded a low day-to-day mean coefficients of variation and high reliability for the bench press (1.1%, intra-class, r = 0.99).

Wingate Anaerobic Capacity Testing: Wingate testing was assessed using the same ergometer as Study 1 and work rate was set at of 7.5 J/kg/rev. Participants were asked to pedal as fast as possible prior to application of the workload and sprint at an all-out maximal capacity for 30-sec Wingate test. Test-to-test variability in performing repeated Wingate anaerobic capacity tests in our lab have yielded correlation coefficients of r = 0.98±15% for mean power.

Food Frequency: All food logs were entered and analyzed by a registered dietitian using dietary analysis software (*ESHA Food Processor Version 8.6, Salem, OR*).

Training Volume: Total lifting volume was calculated for each subject per exercise session and for the entire training program.

Side Effects: The side effects questionnaire was completed every week of supplementation during chronic supplementation (Table 6). The questionnaire was completed to determine how well participants tolerated supplementation; how well participants followed the supplementation protocol; and if participants experienced any symptoms during the supplementation period. Participants were asked to rank the frequency and severity of their symptoms – dizziness, headache, fast or racing heart rate, heart skipping or palpitations, shortness of breath, nervousness, blurred vision, and

unusual or adverse effects. Participants were asked to rank their symptoms with 0 (none), 1 (minimal: 1-2/wk), 2 (slight: 3-4/wk), 3 (occasional: 5-6/wk), 4 (frequent: 7-8/wk), or 5 (severe: 9 or more/wk).

Table 6. Study 2 Side Effects Questionnaire

Week	1	2	3	4	5	6	7	8
Are you training on schedule?								
Are you supplementing on schedule?								
Rate the ***frequency*** of the following symptoms according to the scale where: 0 = none 1 = minimal (1-2 per/wk) 2 = slight (3-4 per/wk) 3 = occasional (5-6 per/wk) 4 = frequent (7-8 per/wk) 5 = severe (9 or more per/wk)								
Dizziness?								
Headache?								
Fast or racing heart rate?								
Heart skipping or palpitations?								
Shortness of breath?								
Nervousness?								
Blurred Vision?								
Any other unusual or adverse effects?								
Rate the ***severity*** of the following symptoms according to the scale where: 0 = none 1 = minimal 2 = slight 3 = moderate 4 = severe 5 = very severe								
Dizziness?								
Headache?								
Fast or racing heart rate?								
Heart skipping or palpitations?								
Shortness of breath?								
Nervousness?								
Blurred Vision?								
Any other unusual or adverse effects?								

Statistical Analysis

Study 1

The primary outcomes included readiness to perform and cognitive function. Secondary outcomes included all respective indices of resistance and Wingate exercise performance testing. Tertiary outcomes included REE, HR, BP and all hematological markers obtained over the testing period. In REE, area under the curve (AUC) of $\dot{V}O_2$, $\dot{V}CO_2$, and RER were calculated by summing up response by every 1-min for 30-min post-ingestion of supplement. AUC were computed on REE data by Prism 6 (*GraphPad Software Inc., La Jolla, CA, USA*) calculating the trapezoid rule and analyzed by ANOVA. All data were analyzed using general linear model Multivariate Analysis of Variance (MANOVA) with Wilks Lambda and Greenhouse Geisser adjustments by the statistical software SPSS 22.0 (*IBM Corporation, Armonk, NY, USA*). Between group comparisons were made using a Dunnet-Hsu post-hoc assessment vs. the PLA condition and all data are presented as mean ± SD. Data were considered significantly different when the probability of error was 0.05 or less with trends noted when p-levels ranged between $p > 0.05$ to $p < 0.05$.

Study 2

The primary outcomes included all respective indices of strength and Wingate anaerobic performance testing. Secondary outcomes included measurements assessing readiness to perform and cognitive function. Tertiary outcomes included resting heart rate, resting blood pressure, and all hematological markers obtained over the testing period. With regard to hematology, we also pursued analyses denoting changes from normal to

exceeding normal clinical limits from baseline to week 4, baseline to week 8 and week 4 to week 8 using a Chi-square analysis. All data were analyzed using general linear models with Wilks Lambda and Greenhouse Geisser adjustments by the statistical software SPSS 22.0. Between group comparisons were made using a Dunnet-Hsu post-hoc assessment vs. the PLA condition and all data are presented as mean ± SD or mean change and 95% CI.

CHAPTER IV

RESULTS

Study 1: Acute Supplementation

Participant Demographics

Figure 7 presents a CONSORT schematic for Study 1. 26 participants were initially recruited for Study 1, completed consent forms, and participated in the required familiarization session. Of the original 26 participants, 25 participants completed Study 1, one participant dropped out after the first testing session due to deny of testing. Table 7 presents Study 1 participant demographics.

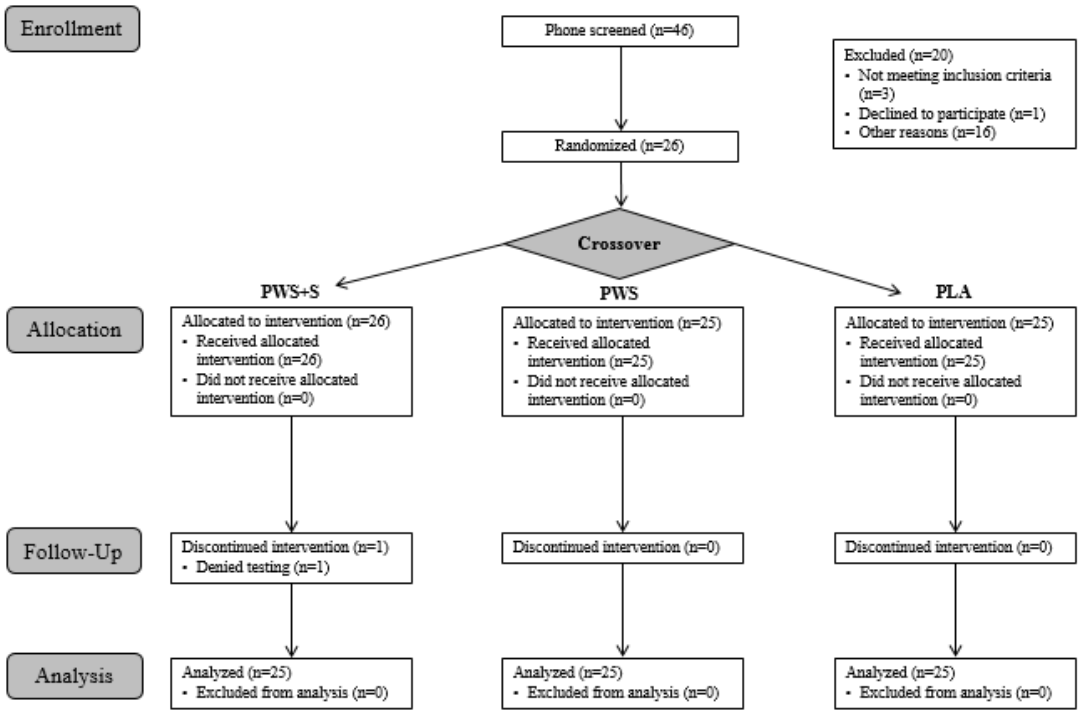

Figure 7. Study 1 - Consort Schematic of Enrollment and Treatment Allocation

Table 7. Study 1 - Participant Demographics

N	Age (y)	Height (cm)	Body Weight (kg)	BMI (kg/m^2)	Lean Mass (kg)	Body Fat (%)
25	21.7 ± 3.0	176.1 ± 8.2	78.2 ± 13.09	25.0 ± 3.0	58.7 ± 11.1	15.2 ± 5.2

Metabolic Responses

Figure 8 shows minute-by-minute comparison of (a) $\dot{V}O_2$, (b) $\dot{V}CO_2$, and (c) RER, for 30-min post-supplementation. Overall, we observed minor, yet significant changes in AUC results for oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), as well as RER averaged over the last 5-min of the REE assessment.

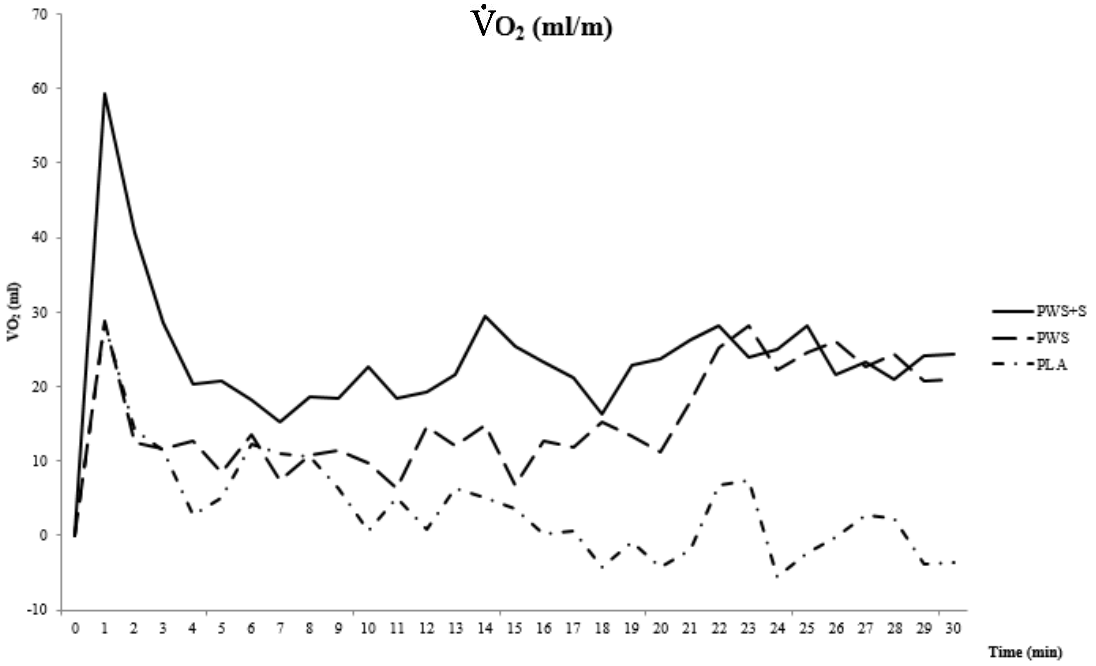

Figure 8a. Study 1 – Minute-by-minute Comparison of $\dot{V}O_2$ Change

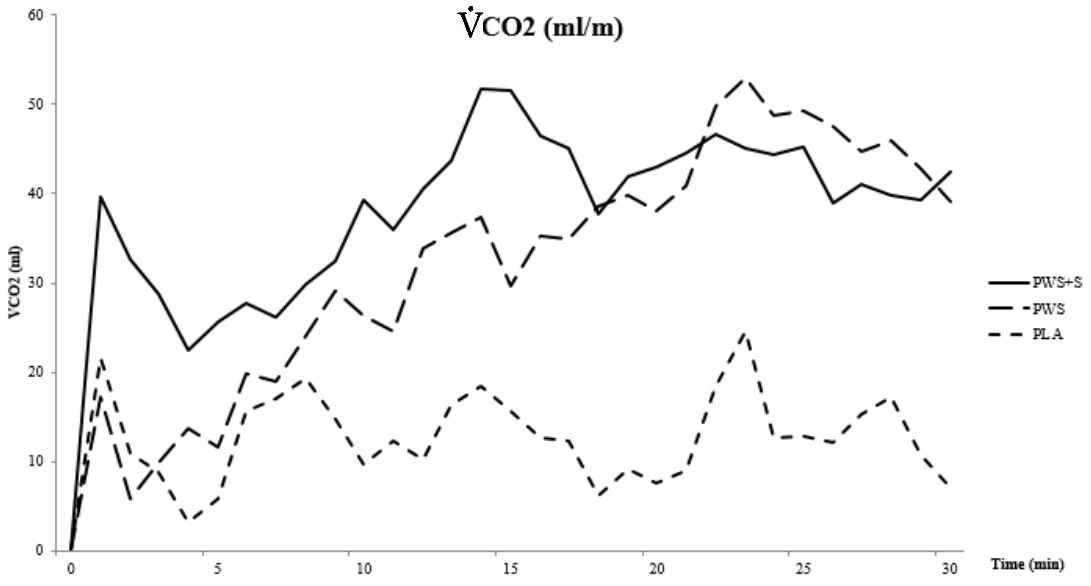

Figure 8b. Study 1 – Minute-by-minute Comparison of $\dot{V}CO_2$ Change

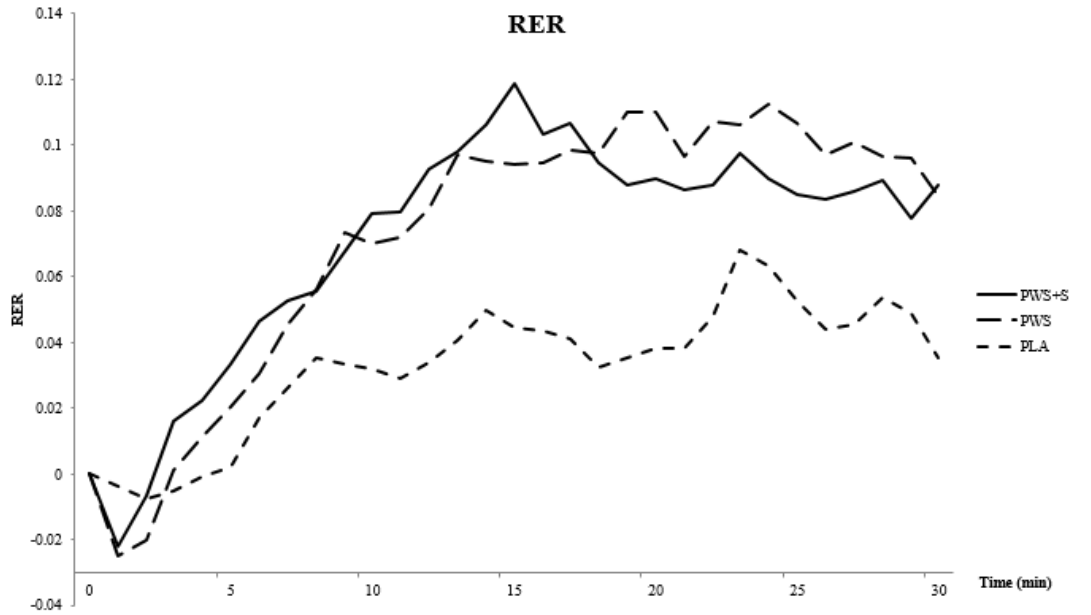

Figure 8c. Study 1 – Minute-by-minute Comparison of RER Change

The AUC for $\dot{V}O_2$ was PWS+S (1,034 ± 584 ml/30min), PWS (802 ± 434 ml/30min), and PLA (684 ± 376 ml/30min) (Fig. 9a). The AUC for $\dot{V}CO_2$ was PWS+S (1,372 ± 604 ml/30min), PWS (1,151 ± 673 ml/30min), and PLA (634 ± 262 ml/30min) (Fig. 9b). RER averaged over the last 5min of the REE assessment showed several significant differences vs. PLA (Fig. 9c). Specifically, the PLA (0.89 ± 0.09) was different from PWS (0.92 ± 0.06, $p < 0.02$) and PWS+S (0.85 ± 0.04, $p = 0.006$) and PWS was significantly different to PWS+S ($p < 0.001$). Despite these perturbations in resting metabolism, no significant differences were noted for REE throughout the 30min measurement period: PWS+S (39.65 ± 9.31 kcal/30min), PWS (40.18 ± 8.97 kcal/30min) and PLA (39.13 ± 5.78 kcals/30min). The results from the $\dot{V}O_2$, $\dot{V}CO_2$, and RER analysis provides supporting evidence which accepted the null hypothesis of hypothesis 1 which stated that there will be significant differences of $\dot{V}O2$, $\dot{V}CO2$, and RER among groups for 30-min post-ingestion.

Cognitive Function and Readiness to Perform

Table 8 and Table 9 show cognitive function and readiness to perform results, respectively. Significant increases were observed in response to "*I feel vigorous and energetic*" for the PWS ($p = 0.02$), PWS+S ($p = 0.02$) but not for the PLA ($p = 0.80$). Also, we found that the mean change in "*I am optimistic about my future performance*" ($p = 0.02$), and in "*I feel vigorous and energetic*" ($p < 0.01$) were significantly increased one hour following supplementation. The results from the cognitive function and readiness to perform test analysis provides supporting evidence which accepted the null hypothesis of hypothesis 2 and hypothesis 3 which stated that there will be significant differences of

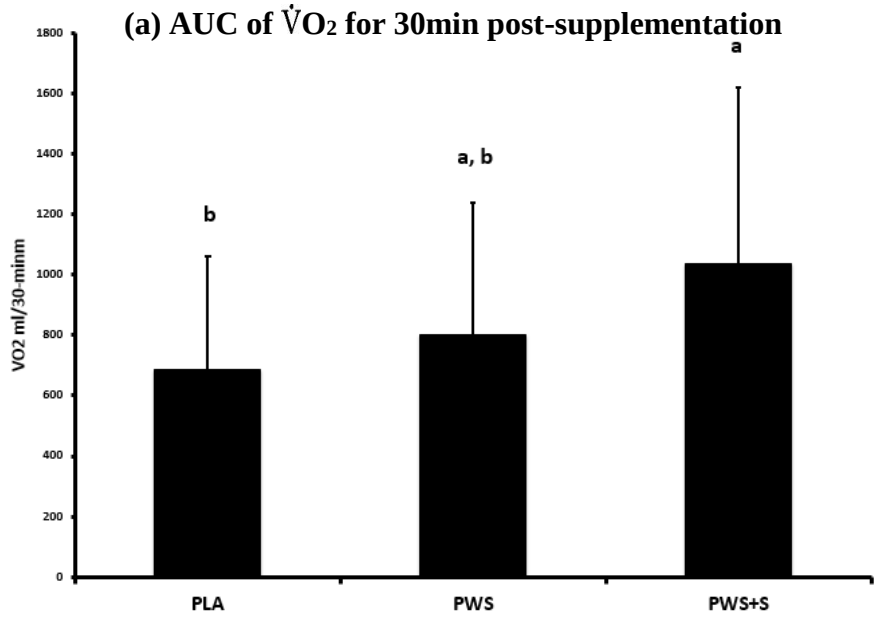

Figure 9a. Study 1 – Area Under the Curve of $\dot{V}O_2$. Data are mean ± SD.

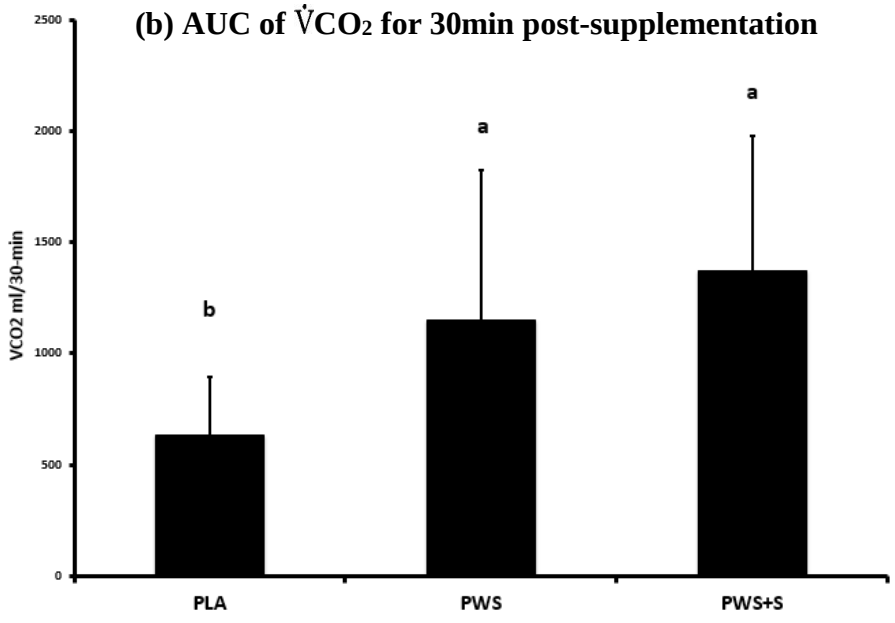

Figure 9b. Study 1 – Area Under the Curve of $\dot{V}CO_2$. Data are mean ± SD.

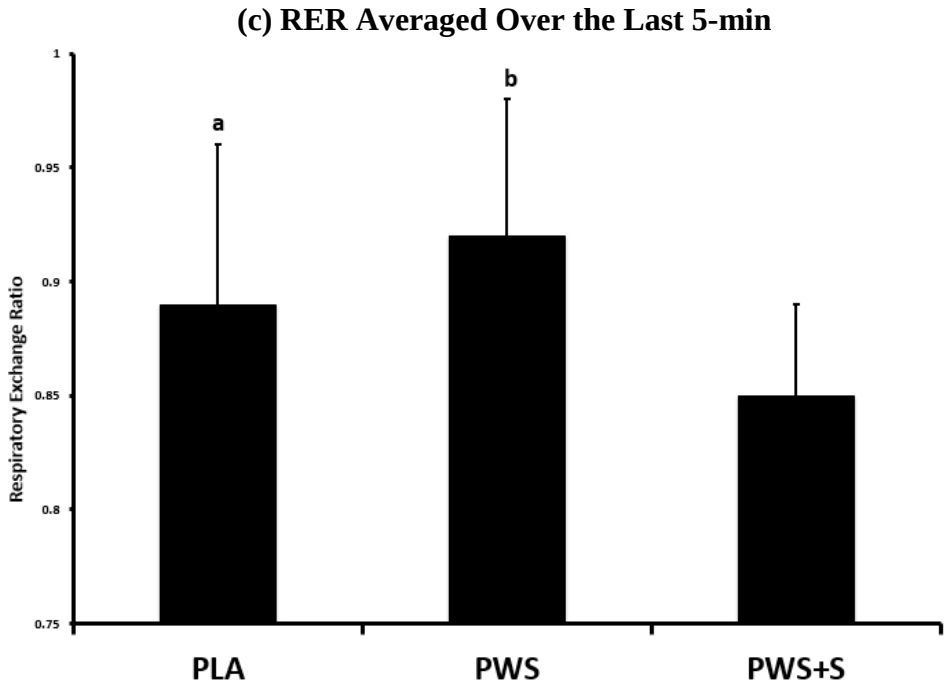

Figure 9c. Study 1 – RER averaged over the last 5-min. Data are mean ± SD. Statistical notations represent - a Significantly different from PWS ($p < 0.02$) and PWS+S ($p = 0.006$) and b significantly different from PWS+S ($p < 0.001$).

cognitive function with Stroop Word-Color test prior to, during, and post-supplementation and there will be significant differences of RTP-VAS among groups prior to, during, and post-supplementation, respectively.

Exercise Performance

We did not observe any significant differences for bench press lifting volume (PLA: 2,166 ± 718; PWS: 2,217 ± 732; PWS+S: 2,268 ± 809 kg; $p = 0.10$) and leg press lifting volume (PLA: 12,550 ± 4,321; PWS: 13,139 ± 4,409; PWS+S: 12,701 ± 3,866 kg; $p = 0.30$). The results from the bench and leg press test analysis provides supporting evidence

Table 8. Study 1 - Stroop Word-Color Test

Variable	Group	Time (h)			Mean ± SE		p-level
		0	1	2			
Word (counts)	PWS+S	104.8 ± 11.4 [b,c]	115.0 ± 13.6 [b,c]	116.8 ± 14.4 [b,c]	112.2 ± 2.4 [b,c]	Group	< 0.001
	PWS	116.7 ± 15.5 [a,c]	119.5 ± 15.4 [a]	124.6 ± 17.7 [a]	120.2 ± 3.0 [a]	Time	< 0.001
	PLA	120.9 ± 15.7 [a,b]	121.1 ± 16.6 [a]	122.6 ± 18.0 [a]	121.5 ± 3.1 [a]	G x T	< 0.001
	Mean ± SE	114.1 ± 2.6	118.5 ± 2.9 *	121.3 ± 3.1 *^			
Color (counts)	PWS+S	79.7 ± 10.5 [b,c]	84.9 ± 11.7 [b,c]	89.8 ± 12.5 [b]	84.8 ± 2.2 [b,c]	Group	0.01
	PWS	91.2 ± 21.3 [a]	95.1 ± 19.9 [a]	99.4 ± 21.0 [a]	95.2 ± 4.0 [a]	Time	< 0.001
	PLA	89.9 ± 12.4 [a]	90.2 ± 13.6 [a]	93.8 ± 17.1	91.3 ± 2.7 [a]	G x T	0.02
	Mean ± SE	86.9 ± 2.4	90.1 ± 2.3 *	94.3 ± 2.7 *^			
Word-Color (counts)	PWS+S	52.4 ± 10.6 [b,c]	57.0 ± 10.4 [b,c]	64.0 ± 10.8 [b,c]	57.8 ± 1.9 [b,c]	Group	< 0.001
	PWS	67.5 ± 20.3 [a]	70.2 ± 21.6 [a]	74.6 ± 18.9 [a]	70.8 ± 4.0 [a]	Time	< 0.001
	PLA	66.4 ± 9.6 [a]	66.8 ± 14.9 [a]	71.3 ± 11.7 [a]	68.2 ± 2.2 [a]	G x T	0.03
	Mean ± SE	62.1 ± 2.1	64.7 ± 2.4 *	69.9 ± 1.9 *^			

Values are means ± standard deviations. Word, Color, and Word-Color counts were analyzed by MANOVA. MANOVA analysis revealed overall Wilks' Lambda group ($p<0.001$), time ($p<0.001$), and group x time ($p<0.001$). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. [a] denotes a significant difference from PWS+S. [b] denotes a significant difference from PWS. [c] denotes a significant difference from PLA. * represents $p<0.05$ difference from baseline. ^ represents $p<0.05$ difference from 1-hr.

Table 9. Study 1 - Readiness to Perform Visual Analogue Scale

Questions	Group	Time (h)			Mean ± SE		p-level
		0	1	2			
I slept well last night	PWS+S	3.51 ± 0.73	3.52 ± 0.73	3.63 ± 0.74	3.55 ± 0.14	Group	0.23
	PWS	3.47 ± 0.61	3.63 ± 0.56	3.59 ± 0.67	3.57 ± 0.11	Time	0.29
	PLA	3.35 ± 0.72	3.33 ± 0.72	3.31 ± 0.84	3.33 ± 0.14	G x T	0.17
	Mean ± SE	3.44 ± 0.09	3.49 ± 0.10	3.51 ± 0.12			
I am looking forward to today's workout	PWS+S	3.71 ± 0.99	3.82 ± 1.17	3.51 ± 1.09	3.68 ± 0.20	Group	0.61
	PWS	3.71 ± 0.77	3.89 ± 0.79	3.65 ± 0.65	3.75 ± 0.13	Time	0.04
	PLA	3.67 ± 0.85	3.61 ± 1.00	3.61 ± 0.87	3.63 ± 0.17	G x T	0.20
	Mean ± SE	3.69 ± 0.15	3.77 ± 0.17	3.87 ± 0.13 ^			
I am optimistic about my future performance	PWS+S	4.00 ± 0.69	4.21 ± 0.62	4.01 ± 0.71	4.07 ± 0.12 [c]	Group	0.01
	PWS	3.88 ± 0.69	4.05 ± 0.73	3.83 ± 0.70	3.92 ± 0.12	Time	0.03
	PLA	3.74 ± 0.83	3.70 ± 0.94	3.78 ± 0.88	3.74 ± 0.17 [a]	G x T	0.19
	Mean ± SE	3.87 ± 0.13	3.99 ± 0.13 *	3.87 ± 0.13			
I feel vigorous and energetic	PWS+S	3.33 ± 0.77	3.89 ± 0.73 [c]	2.96 ± 0.97 [b,c]	3.39 ± 0.13	Group	0.55
	PWS	3.19 ± 0.89	3.77 ± 0.78 [c]	3.33 ± 1.09 [a]	3.43 ± 0.14	Time	0.03
	PLA	3.23 ± 0.95	3.35 ± 0.90 [a,b]	3.38 ± 1.04 [a]	3.32 ± 0.17	G x T	<0.001
	Mean ± SE	3.25 ± 0.16	3.67 ± 0.12 *	3.22 ± 0.18 ^			
My appetite is great	PWS+S	3.80 ± 0.98	3.79 ± 0.89	3.57 ± 0.97	3.72 ± 0.16	Group	0.32
	PWS	3.63 ± 0.86	3.49 ± 0.91	3.43 ± 0.83	3.52 ± 0.14	Time	0.36
	PLA	3.73 ± 0.85	3.55 ± 0.86	3.57 ± 0.87	3.62 ± 0.14	G x T	0.43
	Mean ± SE	3.72 ± 0.15	3.61 ± 0.15	3.52 ± 0.15			
I have little muscle soreness	PWS+S	3.13 ± 1.31	3.27 ± 1.28	3.21 ± 1.12	3.20 ± 0.21	Group	0.16
	PWS	3.54 ± 1.17	3.81 ± 1.35	3.25 ± 1.03	3.53 ± 0.21	Time	0.17
	PLA	3.60 ± 0.99	3.42 ± 0.99	3.29 ± 1.01	3.44 ± 0.16	G x T	0.16
	Mean ± SE	3.43 ± 0.17	3.50 ± 0.21	3.25 ± 0.18			

Values are means ± standard deviations. Six questions were analyzed by MANOVA. MANOVA analysis revealed overall Wilks' Lambda group (p=0.03), time (p=0.04), and group x time (p=0.007). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. [a] denotes a significant difference from PWS+S. [b] denotes a significant difference from PWS. [c] denotes a significant difference from PLA. * represents p<0.05 difference from baseline. ^ represents p<0.05 difference from 1-hr.

which rejected the null hypothesis of hypothesis 4 and hypothesis 5 which stated that there will be significant differences of upper body strength with bench press test among groups after supplementation and there will be significant differences of lower body strength with leg press test among groups after supplementation, respectively.

Similarly, no significant differences were found in between groups Wingate peak power (PLA: 19.89 ± 5.14; PWS: 19.03 ± 5.29; PWS+S: 18.86 ± 5.22 Watt/kg; $p = 0.51$), mean power (PLA: 7.68 ± 1.08; PWS: 7.66 ± 1.27; PWS+S: 7.76 ± 1.33 Watt/kg; $p = 0.77$), or total work (PLA: 17,662 ± 4,605; PWS: 17,850 ± 4,340; PWS+S: 18,203 ± 4,658 Joules; $p = 0.49$). The results from the Wingate anaerobic sprint test analysis provides supporting evidence which rejected the null hypothesis of hypothesis 6 which stated that there will be significant differences of anaerobic capacity with WAT among groups after supplementation.

Blood Pressure and Heart Rate Responses

No significant treatment effects over 30 minutes were observed from un-supplemented baseline HR: PWS+S (57.9 ± 1.3 b/min), PWS (56.5 ± 1.3 b/min), PLA (59.5 ± 1.4 b/min), systolic blood pressure PWS+S (113.4 ± 1.6 mmHg), PWS (112.4 ± 1.4 mmHg), PLA (113.6 ± 1.2 mmHg) or diastolic blood pressure PWS+S (70.0 ± 1.3 mmHg), PWS (70.3 ± 1.3 mmHg), PLA (69.2 ± 1.2 mmHg) following treatment ingestion (Table 10). The results from the HR, SBP, and DBP test analysis provides supporting evidence which accepted the null hypothesis of hypothesis 7 which stated that there will be no significant differences of resting heart rate and blood pressure among groups prior to, during, and post-supplementation.

Hematologic Responses

Results of blood analyses are presented in Table 11. Overall, we observed several significant changes in various blood markers during the 2-h post-supplementation following a series of exercise tests; however, the changes as a whole were random in nature and inconsistent amongst and between groups. The results from the blood chemistry test analysis provides supporting evidence which rejected the null hypothesis of hypothesis 8 and 10 which stated that there will be no significant differences of blood metabolic markers; cholesterol, HDL-C, LDL-C, glucose among groups prior to and 2-hr post supplementation, and there will be no significant differences of kidney enzymes, CRE and BUN among groups prior to and 2-hr post supplementation, respectively. In contrast, the results from the blood chemistry test analysis provides supporting evidence which accepted the null hypothesis of hypothesis 9 and 11 which stated that there will be no significant differences of muscle enzymes, LDH and CK among groups prior to and 2-hr post supplementation, and there will be no significant differences of liver enzymes; ALP, ALT, and AST among groups prior to and 2-hr post supplementation, respectively.

Table 10. Study 1 - Heart Rate and Blood Pressure Response

Variable	Group	Time (min)					Mean ± SE		p-level
		Pre	0	10	20	30			
HR (beats/min)	PWS+S	56.6 ± 7.2	57.5 ± 8.8	56.5 ± 9.5	58.4 ± 9.1	60.4 ± 10.8	57.9 ± 1.3	Group	0.11
	PWS	55.5 ± 7.3	58.2 ± 9.5	54.2 ± 7.2	56.5 ± 7.7	58.1 ± 8.1	56.5 ± 1.3	Time	< 0.001
	PLA	58.2 ± 9.5	60.4 ± 8.1	58.6 ± 8.5	59.6 ± 8.3	60.6 ± 8.4	59.5 ± 1.4	G x T	0.83
	Mean ± SE	56.8 ± 1.1	58.7 ± 1.2 *	56.4 ± 1.3 ^	58.2 ± 1.1	59.7 ± 1.3 *#			
SBP (mmHg)	PWS+S	113.1 ± 7.8	112.5 ± 8.3	113.0 ± 9.6	113.9 ± 10.6	114.6 ± 10.0	113.4 ± 1.6	Group	0.55
	PWS	111.5 ± 7.1	112.4 ± 7.3	112.9 ± 8.6	112.8 ± 7.9	112.5 ± 8.2	112.4 ± 1.4	Time	0.31
	PLA	113.3 ± 7.6	114.3 ± 8.0	112.7 ± 6.8	114.3 ± 6.3	113.6 ± 6.6	113.6 ± 1.2	G x T	0.52
	Mean ± SE	112.6 ± 1.2	113.1 ± 1.3	112.8 ± 1.3	113.6 ± 1.3	113.5 ± 1.3			
DBP (mmHg)	PWS+S	69.6 ± 7.2	70.0 ± 6.7	71.0 ± 6.8	71.1 ± 8.1	68.4 ± 13.0	70.0 ± 1.3	Group	0.76
	PWS	69.0 ± 6.7	70.7 ± 6.1	70.9 ± 7.1	70.6 ± 7.5	70.4 ± 7.7	70.3 ± 1.3	Time	0.45
	PLA	69.6 ± 6.8	69.2 ± 6.5	68.8 ± 6.3	69.5 ± 7.4	69.1 ± 7.1	69.2 ± 1.2	G x T	0.50
	Mean ± SE	69.4 ± 0.8	69.9 ± 0.8	70.2 ± 1.0	70.4 ± 1.1	69.3 ± 1.2			

Values are means ± standard deviations. Heart Rate (HR), Systolic Blood Pressure (SBP), and Diastolic Blood Pressure (DBP) were analyzed by MANOVA. MANOVA analysis revealed overall Wilks' Lambda group (p=0.27), time (p=0.03), and group x time (p=0.81). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. [a] denotes a significant difference from PWS+S. [b] denotes a significant difference from PWS. [c] denotes a significant difference from PLA. * represents p<0.05 difference from pre-supplementation. ^ represents p<0.05 difference from 0-min. [#] represents p<0.05 difference from 10-min.

Table 11. Study 1 - Hematological Response

Variable	Group	Time (h)		Mean ± SE		p-level
		0	2			
ALP	PWS+S	75.0 ± 19.2	82.2 ± 23.0	78.6 ± 4.2	Group	0.27
(U/L)	PWS	77.7 ± 31.1	82.3 ± 22.5	80.0 ± 5.3	Time	< 0.001
	PLA	79.8 ± 21.7	85.7 ± 27.5	82.7 ± 4.9	G x T	0.69
	Mean ± SE	77.5 ± 4.5	83.4 ± 4.7 *			
ALT	PWS+S	25.7 ± 11.7	27.1 ± 10.1	26.4 ± 2.0	Group	0.43
(U/L)	PWS	24.5 ± 11.0	28.0 ± 12.2	26.2 ± 2.3	Time	< 0.001
	PLA	29.5 ± 27.6	33.3 ± 32.9	31.4 ± 6.1	G x T	0.26
	Mean ± SE	26.6 ± 2.5	29.5 ± 2.8 *			
AST	PWS+S	25.3 ± 6.6	28.8 ± 6.5	27.1 ± 1.3	Group	0.31
(U/L)	PWS	24.9 ± 6.2	29.6 ± 7.1	27.3 ± 1.3	Time	< 0.001
	PLA	38.1 ± 60.6	44.9 ± 75.7	41.5 ± 13.9	G x T	0.37
	Mean ± SE	29.5 ± 4.2	34.4 ± 5.2 *			
BUN	PWS+S	16.4 ± 4.0	15.9 ± 3.5	16.1 ± 0.7	Group	0.22
(mg/dl)	PWS	17.4 ± 4.7	15.1 ± 3.7	16.2 ± 0.8	Time	< 0.001
	PLA	15.9 ± 4.8	14.4 ± 4.1	15.1 ± 0.9	G x T	0.01
	Mean ± SE	16.6 ± 0.7	15.1 ± 0.6 *			
Creatinine	PWS+S	0.97 ± 0.14 [b]	1.11 ± 0.17 [b,c]	1.04 ± 0.03 [b]	Group	< 0.001
(mg/dl)	PWS	1.09 ± 0.17 [a,c]	1.23 ± 0.17 [a,c]	1.16 ± 0.03 [a,c]	Time	< 0.001
	PLA	0.99 ± 0.14 [b]	1.05 ± 0.16 [a,b]	1.02 ± 0.03 [b]	G x T	0.03
	Mean ± SE	1.02 ± 0.02	1.13 ± 0.03 *			
BUN:Creatinine	PWS+S	17.1 ± 4.8	14.4 ± 3.4 [c]	15.7 ± 0.8	Group	0.18
	PWS	16.2 ± 5.0	12.3 ± 3.2 [a,c]	14.3 ± 0.8	Time	< 0.001
	PLA	16.2 ± 5.9	13.9 ± 4.9 [b]	15.1 ± 1.1	G x T	< 0.001
	Mean ± SE	16.5 ± 0.9	13.5 ± 0.6 *			

Values are means ± standard deviations. ALP, ALT, AST, BUN, Creatinine, ratio of BUN to Creatinine, Cholesterol, HDL-C, ratio of Cholesterol to HDLC, LDL-C, Triglyceride, CK, LDH, and Glucose were analyzed by MANOVA. MANOVA analysis revealed overall Wilks' Lambda group ($p<0.001$), time ($p<0.001$), and group x time ($p<0.001$). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. [a] denotes a significant difference from PWS+S. [b] denotes a significant difference from PWS. [c] denotes a significant difference from PLA. * represents $p<0.05$ difference from baseline.

Table 11. Continued

Variable	Group	Time (h)		Mean ± SE		p-level
		0	2			
Cholesterol	PWS+S	161.7 ± 29.8 [b,c]	174.0 ± 33.5 [c]	167.8 ± 6.3 [b,c]	Group	< 0.001
(mg/dl)	PWS	187.3 ± 46.6 [a,c]	180.2 ± 36.9	183.7 ± 8.1 [a]	Time	< 0.001
	PLA	170.6 ± 34.6 [a,b]	182.8 ± 35.1 [a]	176.7 ± 7.0 [a]	G x T	< 0.001
	Mean ± SE	173.2 ± 6.7	179.0 ± 6.9 *			
HDL-C	PWS+S	54.2 ± 16.2	60.1 ± 18.1	57.2 ± 3.4	Group	0.50
(mg/dl)	PWS	52.2 ± 13.9	58.9 ± 14.6	55.5 ± 2.9	Time	< 0.001
	PLA	54.9 ± 13.3	60.1 ± 16.0	57.5 ± 2.8	G x T	0.57
	Mean ± SE	53.8 ± 2.7	59.7 ± 3.1 *			
CHOL:HDL	PWS+S	3.2 ± 1.0 [b]	3.1 ± 1.0	3.1 ± 0.2 [b]	Group	< 0.001
	PWS	3.8 ± 1.3 [a,c]	3.2 ± 1.0	3.5 ± 0.2 [a,c]	Time	< 0.001
	PLA	3.2 ± 0.9 [b]	3.2 ± 0.9	3.2 ± 0.1 [b]	G x T	< 0.001
	Mean ± SE	3.4 ± 0.2	3.1 ± 0.1 *			
LDL-C	PWS+S	99.8 ± 41.2	104.6 ± 40.7	102.2 ± 8.2	Group	0.18
(mg/dl)	PWS	96.9 ± 31.9	104.5 ± 36.0	100.7 ± 6.9	Time	< 0.001
	PLA	104.3 ± 36.7	109.6 ± 41.2	107.0 ± 7.8	G x T	0.56
	Mean ± SE	100.3 ± 7.2	106.3 ± 7.7 *			
Triglyceride	PWS+S	88.3 ± 42.2	92.8 ± 54.6	90.5 ± 9.6	Group	0.25
(mg/dl)	PWS	109.5 ± 56.8	100.3 ± 47.5	104.9 ± 10.1	Time	0.37
	PLA	98.00 ± 58.6	89.3 ± 38.3	93.6 ± 9.3	G x T	0.13
	Mean ± SE	98.6 ± 8.9	94.1 ± 8.2			
CK	PWS+S	189.2 ± 85.4	222.5 ± 84.6	205.9 ± 17.1	Group	0.29
(U/L)	PWS	191.4 ± 104.1	230.8 ± 111.3	211.1 ± 21.9	Time	0.03
	PLA	713.3 ± 2354.3	836.4 ± 2785.6	774.8 ± 524.6	G x T	0.33
	Mean ± SE	364.6 ± 160.4	429.9 ± 190.0 *			
LDH	PWS+S	166.9 ± 25.5	192.2 ± 24.2	179.5 ± 4.8	Group	0.35
(U/L)	PWS	160.65 ± 18.0	186.7 ± 22.2	173.6 ± 3.9	Time	< 0.001
	PLA	181.7 ± 76.8	205.4 ± 114.7	193.5 ± 19.4	G x T	0.87
	Mean ± SE	169.7 ± 6.6	194.8 ± 9.1 *			
Glucose	PWS+S	90.4 ± 7.3 [b]	113.8 ± 15.0 [c]	102.1 ± 1.9 [b,c]	Group	< 0.001
(mg/dl)	PWS	100.1 ± 16.0 [a,c]	114.4 ± 12.6 [c]	107.3 ± 1.9 [a,c]	Time	< 0.001
	PLA	90.2 ± 9.7 [b]	101.5 ± 14.4 [a,b]	95.8 ± 2.0 [a,b]	G x T	0.03
	Mean ± SE	93.6 ± 1.4	109.9 ± 2.2 *			

Values are means ± standard deviations. ALP, ALT, AST, BUN, Creatinine, ratio of BUN to Creatinine, Cholesterol, HDL-C, ratio of Cholesterol to HDLC, LDL-C, Triglyceride, CK, LDH, and Glucose were analyzed by MANOVA. MANOVA analysis revealed overall Wilks' Lambda group ($p<0.001$), time ($p<0.001$), and group x time ($p<0.001$). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. a denotes a significant difference from PWS+S. b denotes a significant difference from PWS. c denotes a significant difference from PLA. * represents $p<0.05$ difference from baseline.

Study 2: Chronic Supplementation

Participant Demographics

Figure 10 presents a CONSORT schematic for Study 2. 122 participants were initially recruited for Study 2, completed consent forms, and participated in the required familiarization session. Of the original 122 participants, 80 participants completed Study 2. Table 12 presents Study 2 participant demographics.

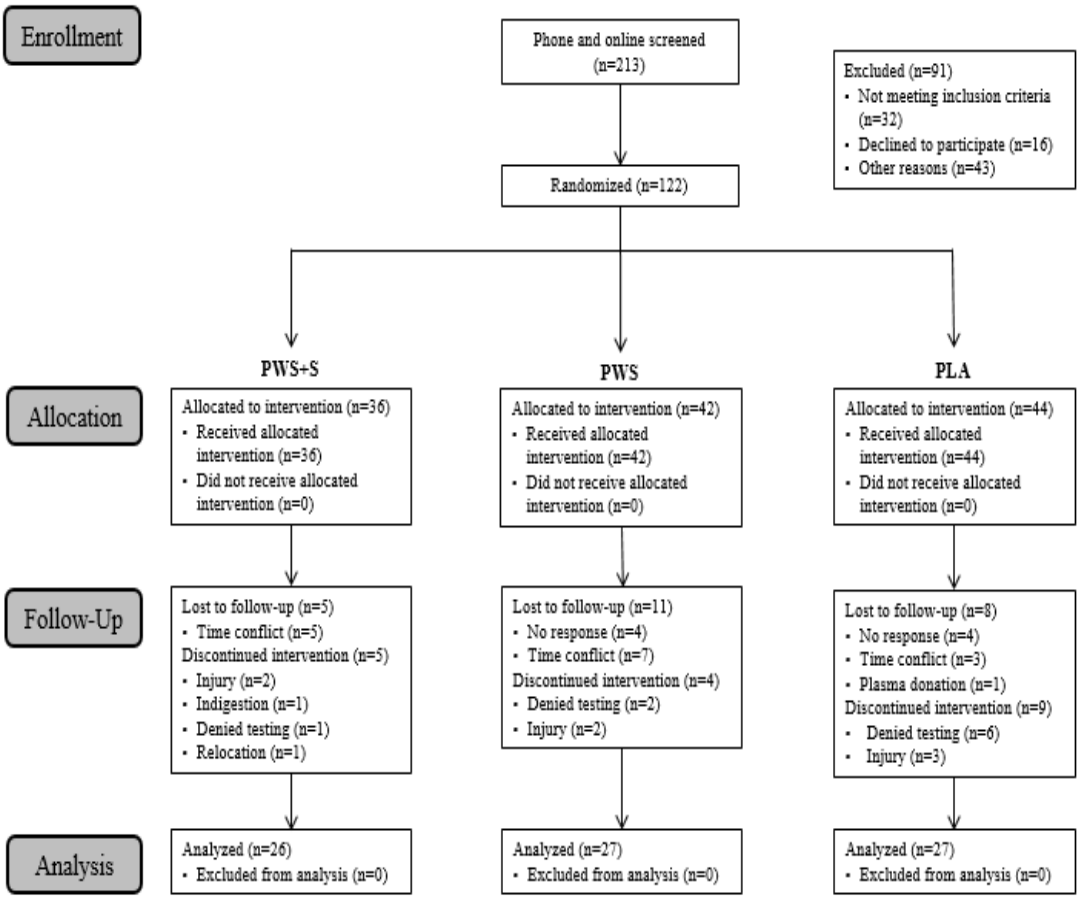

Figure 10. Study 2 Consort Schematic of Enrollment and Treatment Allocation

Table 12. Study 2 - Participant Demographics

Variable	Group	N	Values	p-level
Age (y)	PWS+S	26	22.0 ± 2.6	0.31
	PWS	27	20.9 ± 3.9	
	PLA	27	22.3 ± 3.9	
Height (cm)	PWS+S	26	177.8 ± 5.6	0.64
	PWS	27	177.0 ± 4.6	
	PLA	27	178.4 ± 6.9	
Body Weight (kg)	PWS+S	26	80.2 ± 15.8	0.94
	PWS	27	81.5 ± 13.0	
	PLA	27	81.1 ± 13.3	
BMI (kg/m^2)	PWS+S	26	25.4 ± 3.4	0.72
	PWS	27	26.1 ± 4.6	
	PLA	27	25.4 ± 3.4	

Values are means ± standard deviations. Variables were analyzed by one-way ANOVA.

Training and Dietary Characteristics

Table 13 presents total training volume of upper and lower body for 8-wk. No significant differences were noted for total training volume over the eight-week study period: PLA (550321 ± 287503 kg), PWS (520515 ± 169245 kg) PWS+S (509392 ± 280566 kg). The results from the training volume analysis provides supporting evidence which accepted the null hypothesis of hypothesis 12 which stated that there will be no significant differences of training volume of upper and lower body among groups over 8-wk following supplementation.

Participants presented to the study consuming a total of 2280.20 ± 783.86 kcals/day, partitioned for 128.77 ± 54.47 g/d of protein, 210.58 ± 77.09 g/d of carbohydrate and 91.90 ± 41.08 g/d of fat averaged over 8-wk study period. No significant changes in diet were observed during the study period (Table 14). The results from the

dietary consumption analysis provides supporting evidence which accepted the null hypothesis of hypothesis 13 which stated that there will be no significant differences of consumption of macronutrient, CHO, FAT, and PRO, among groups over 8-wk following supplementation.

Table 13. Study 2 - Total Workout Volume for 8 Week

Variable	Group	N	Total Volume	p-level
Upper body (kg)	PWS+S	23	269928 ± 103130	0.32
	PWS	27	236691 ± 87062	
	PLA	25	279831 ± 132101	
Lower body (kg)	PWS+S	23	305906 ± 133611	0.65
	PWS	27	283825 ± 100541	
	PLA	25	314516 ± 136966	

Values are means ± standard deviations. Total training volume was analyzed by one-way MANOVA. MANOVA analysis revealed overall Wilks' Lambda group (p=0.69). p-levels was reported with between-subjects effects.

Body Composition

Table 15 shows the results from all body composition analysis. A MANOVA analysis was used to examine changes in body composition variables included body weight, fat mass, fat-free mass, and percentage of body fat. The results from the DXA analysis provides supporting evidence which rejected the null hypothesis of hypothesis 14 which stated that there will be significant differences of fat mass with DXA body scan among groups over 8 weeks following supplementation.

Table 14. Study 2 - Dietary Characteristics

Variable	Group	N	Time (wk)			Mean ± SE		p-level
			0	4	8			
Calories (kcal/d)	PWS+S	23	2277.0 ± 755.6	2285.8 ± 904.4	2514.2 ± 1129.0	2359.0 ± 140.3	Group	0.21
	PWS	25	2464.5 ± 786.6	2083.1 ± 653.4	2178.3 ± 1025.4	2241.9 ± 134.6	Time	0.24
	PLA	26	2124.6 ± 706.6	2011.0 ± 590.4	1944.3 ± 769.3	2026.6 ± 132.0	G x T	0.17
	Mean ± SE		2288.7 ± 87.2	2126.7 ± 83.9	2212.3 ± 113.9			
Protein (g/d)	PWS+S	23	135.9 ± 57.8	140.7 ± 79.9	138.2 ± 67.4	138.3 ± 10.4	Group	0.34
	PWS	25	128.3 ± 49.6	112.8 ± 43.4	127.1 ± 79.8	122.7 ± 9.9	Time	0.76
	PLA	26	117.8 ± 41.5	117.5 ± 49.0	118.3 ± 49.6	117.9 ± 9.7	G x T	0.68
	Mean ± SE		127.3 ± 5.7	123.7 ± 6.8	127.9 ± 7.7			
Carbohydrate (g/d)	PWS+S	23	203.1 ± 64.3	206.1 ± 95.5	195.0 ± 85.0	201.4 ± 13.3	Group	0.84
	PWS	25	231.7 ± 90.6	197.3 ± 76.6	187.7 ± 88.0	205.6 ± 12.8	Time	0.02
	PLA	26	204.9 ± 75.2	204.4 ± 68.7	175.8 ± 68.4	195.0 ± 12.5	G x T	0.38
	Mean ± SE		213.2 ± 9.0	202.6 ± 9.3	186.2 ± 9.3 *			
Fat (g/d)	PWS+S	23	93.6 ± 39.7	93.6 ± 61.2	101.5 ± 49.6	96.2 ± 6.8 [c]	Group	0.80
	PWS	25	100.8 ± 38.9	85.6 ± 38.8	95.1 ± 49.0	93.8 ± 6.5	Time	0.24
	PLA	26	82.3 ± 36.9	70.8 ± 27.1	77.5 ± 43.3	76.9 ± 6.4 [a]	G x T	0.80
	Mean ± SE		92.2 ± 4.4	83.4 ± 5.1	91.4 ± 5.5			

Values are means ± standard deviations. Total calories, Protein, Carbohydrate, and Fat intake were analyzed by MANOVA. MANOVA analysis revealed overall Wilks' Lambda group (p=0.04), time (p=0.03), and group x time (p=0.35). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. * represents p<0.05 difference from baseline. ^ represents p<0.05 difference from wk 4.

Table 14. Continued

Variable	Group	N	Time (wk)			Mean ± SE		p-level
			0	4	8			
Calories (kcal/d/kg)	PWS+S	23	29.03 ± 9.73	29.35 ± 13.75	32.13 ± 16.48	30.17 ± 2.03	Group	0.27
	PWS	25	30.15 ± 8.76	25.72 ± 8.10	26.29 ± 10.99	27.39 ± 1.94	Time	0.29
	PLA	26	27.06 ± 10.94	25.33 ± 9.31	24.72 ± 13.50	25.70 ± 1.90	G x T	0.19
	Mean ± SE		28.75 ± 1.15	26.80 ± 1.22	27.71 ± 1.60			
Protein (g/d/kg)	PWS+S	23	1.74 ± 0.79	1.83 ± 1.13	1.77 ± 0.94	1.78 ± 0.13	Group	0.18
	PWS	25	1.54 ± 0.50	1.37 ± 0.47	1.51 ± 0.84	1.47 ± 0.13	Time	0.86
	PLA	26	1.48 ± 0.57	1.46 ± 0.64	1.49 ± 0.79	1.48 ± 0.12	G x T	0.72
	Mean ± SE		1.59 ± 0.07	1.55 ± 0.09	1.59 ± 0.10			
Carbohydrate (g/d/kg)	PWS+S	23	2.61 ± 0.89	2.64 ± 1.43	2.42 ± 1.17	2.56 ± 0.19	Group	0.91
	PWS	25	2.87 ± 1.10	2.46 ± 1.02	2.30 ± 1.00	2.55 ± 0.18	Time	0.008
	PLA	26	2.60 ± 1.11	2.56 ± 0.95	2.21 ± 1.10	2.46 ± 0.18	G x T	0.55
	Mean ± SE		2.70 ± 0.12	2.55 ± 0.13	2.31 ± 0.12 *^			
Fat (g/d/kg)	PWS+S	23	1.19 ± 0.51	1.19 ± 0.84	1.30 ± 0.71	1.23 ± 0.09	Group	0.14
	PWS	25	1.22 ± 0.41	1.05 ± 0.45	1.14 ± 0.52	1.14 ± 0.08	Time	0.30
	PLA	26	1.05 ± 0.54	0.90 ± 0.41	0.98 ± 0.67	0.98 ± 0.08	G x T	0.79
	Mean ± SE		1.15 ± 0.05	1.05 ± 0.06	1.14 ± 0.07			

Values are means ± standard deviations. Total calories, Protein, Carbohydrate, and Fat intake were analyzed by MANOVA. MANOVA analysis revealed overall Wilks' Lambda group ($p=0.04$), time ($p=0.03$), and group x time ($p=0.35$). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. * represents $p<0.05$ difference from baseline. ^ represents $p<0.05$ difference from wk 4.

Table 15. Study 2 - Body Composition

Variables	Group	N	Time (wk)			Mean ± SE		p-level
			0	4	8			
Body Weight (kg)	PWS+S	26	80.4 ± 16.1	81.3 ± 16.7	81.8 ± 17.3	81.2 ± 2.8	Group	0.98
	PWS	27	81.8 ± 13.3	81.3 ± 11.3	82.2 ± 12.8	81.8 ± 2.7	Time	< 0.01
	PLA	27	81.1 ± 13.4	81.8 ± 14.2	82.1 ± 14.0	81.7 ± 2.7	G x T	0.27
	Mean ± SE		81.1 ± 1.6	81.5 ± 1.5	82.1 ± 1.5 *^			
Fat Mass (kg)	PWS+S	26	11.3 ± 7.3	11.6 ± 8.3	11.5 ± 8.5	11.5 ± 1.3	Group	0.75
	PWS	27	12.7 ± 7.6	13.0 ± 7.7	12.8 ± 7.4	12.8 ± 1.3	Time	0.10
	PLA	27	11.3 ± 5.4	11.9 ± 5.5	11.9 ± 5.2	11.7 ± 1.3	G x T	0.60
	Mean ± SE		11.8 ± 0.7	12.1 ± 0.8 *	12.1 ± 0.8			
Fat-Free Mass (kg)	PWS+S	26	62.4 ± 9.2	63.0 ± 9.0	63.6 ± 9.1	63.0 ± 1.7	Group	0.94
	PWS	27	62.3 ± 7.2	62.2 ± 6.7	62.7 ± 6.6	62.4 ± 1.7	Time	< 0.01
	PLA	27	63.0 ± 10.7	63.2 ± 11.2	63.4 ± 11.0	63.2 ± 1.7	G x T	0.28
	Mean ± SE		62.6 ± 1.0	62.8 ± 1.0	63.2 ± 1.0 *^			
Body Fat (%)	PWS+S	26	14.5 ± 5.8	14.5 ± 6.3	14.3 ± 6.1	14.4 ± 1.2	Group	0.55
	PWS	27	16.1 ± 6.6	16.4 ± 6.7	16.2 ± 6.3	16.2 ± 1.1	Time	0.23
	PLA	27	15.1 ± 6.2	15.7 ± 6.0	15.7 ± 5.6	15.5 ± 1.1	G x T	0.35
	Mean ± SE		15.2 ± 0.7	15.5 ± 0.7	15.4 ± 0.6			

Values are means ± standard deviations. All variables were analyzed by MANOVA. MANOVA analysis revealed overall Wilks' Lambda group (p=0.06), time (p=0.03), and group x time (p=0.29). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. [a] denotes a significant difference from PWS+S. [b] denotes a significant difference from PWS. [c] denotes a significant difference from PLA. * represents p<0.05 difference from baseline. ^ represents p<0.05 difference from wk 4.

Cognitive Function and Readiness to Perform

Table 16 shows the results for our cognitive function testing demonstrating significant within group increases for Stroop Test Word, Color and Word-Color counts. Specific comparisons at week 4 demonstrated as significant increase in Word count for the PLA (3.92 counts, 95% CI 0.39, 7.45) and PWS+S (5.46 counts, 95% CI 2.09, 9.19) group, but not for PWS (3.21 counts, 95% CI -0.31, 6.72). By week 8 all groups increased their respective Word counts: PLA (6.74 counts, 95% CI 3.32, 10.16), PWS (7.56 counts, 95% CI 4.15, 10.97) and PWS+S (9.93 counts, 95% CI 6.49, 13.73). For the Color assessment comparison week 4 changes are: PLA (2.77 counts, 95% CI 0.43, 5.09), PWS (5.05 counts, 95% CI 2.72, 7.38), and PWS+S (2.57 counts, 95% CI 0.24, 4.88). For week 8, Color assessment changes are: PLA (4.90 counts, 95% CI 2.32, 7.46), PWS (8.33 counts, 95% CI 5.76, 10.89) and PWS+S (5.08 counts, 95% CI 2.51, 7.63). For week 4 Word-Color changes were significant for the PWS (3.99 counts, 95% CI 1.75, 6.23), and PWS+S (5.27 counts, 95% CI 3.01, 7.52), but not the PLA (2.08 counts, 95% CI -0.15, 4.31) group. By week 8, all groups demonstrated a significant increase in word-color counts: PLA (5.02 counts, 95% CI 2.44, 7.59), PWS (5.84 counts, 95% CI 3.27, 8.41) and PWS+S (6.13 counts, 95% CI 3.54, 8.72).

However, no between group significance was otherwise noted and no differences were noted for any parameters denoting “Readiness to Perform” via our VAS testing (Table 17). The results from the Stroop Word-Color and RTP-VAS test analysis provides supporting evidence which rejected the null hypothesis of hypothesis 15 and 16 which stated that there will be significant differences of cognitive function with Stroop Word-

Table 16. Study 2 - Stroop Word-Color Testing Assessment

Variable	Group	N	Time (wk)			Mean ± SE		p-level
			0	4	8			
Word (counts)	PWS+S	26	102.3 ± 11.2 [c]	108.2 ± 17.0	112.4 ± 16.1	107.6 ± 2.7	Group	0.33
	PWS	27	107.7 ± 11.4	110.6 ± 10.9	115.3 ± 10.9	111.2 ± 2.6	Time	< 0.001
	PLA	27	109.8 ± 16.9 [a]	113.6 ± 17.2	116.2 ± 17.5	113.2 ± 2.6	G x T	0.45
	Mean ± SE		106.6 ± 1.5	110.8 ± 1.7 *	114.6 ± 1.6 *^			
Color (counts)	PWS+S	26	77.0 ± 10.4	79.7 ± 9.8	82.1 ± 10.8	79.6 ± 1.9	Group	0.35
	PWS	27	78.2 ± 9.4	83.6 ± 9.6	86.7 ± 10.1	82.8 ± 1.9	Time	< 0.001
	PLA	27	80.8 ± 10.6	83.2 ± 11.8	85.7 ± 12.5	83.2 ± 1.9	G x T	0.19
	Mean ± SE		78.7 ± 1.1	82.2 ± 1.7 *	84.9 ± 1.2 *^			
Word-Color (counts)	PWS+S	26	49.2 ± 11.1	55.0 ± 9.6	55.6 ± 10.2	53.3 ± 1.8	Group	0.42
	PWS	27	53.1 ± 5.9	56.7 ± 7.4	59.1 ± 7.6	56.3 ± 1.7	Time	< 0.001
	PLA	27	54.1 ± 11.5	55.5 ± 11.5	58.5 ± 12.6	56.0 ± 1.7	G x T	0.17
	Mean ± SE		52.1 ± 1.0	55.7 ± 1.0 *	57.7 ± 1.1 *^			

Values are means ± standard deviations. Word, Color, and Word-Color counts were analyzed by MANOVA. MANOVA analysis revealed overall Wilks' Lambda group (p=0.83), time (p<0.001), and group x time (p=0.17). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. [a] denotes a significant difference from PWS+S. [b] denotes a significant difference from PWS. [c] denotes a significant difference from PLA. * represents p<0.05 difference from baseline. ^ represents p<0.05 difference from wk 4.

Table 17. Study 2 - Readiness to Perform Visual Analogue Scale

Variable	Group	N	Time (wk)			Mean ± SE		p-level
			0	4	8			
I slept well last night	PWS+S	26	3.21 ± 1.09 [b]	3.26 ± 1.11	3.59 ± 0.91	3.36 ± 0.14	Group	0.25
	PWS	27	3.77 ± 0.93 [a]	3.75 ± 0.89	3.52 ± 1.17	3.68 ± 0.13	Time	0.83
	PLA	27	3.46 ± 0.90	3.43 ± 0.92	3.53 ± 0.90	3.47 ± 0.13	G x T	0.34
	Mean ± SE		3.48 ± 0.11	3.48 ± 0.11	3.55 ± 0.11			
I am looking forward to today's workout	PWS+S	26	3.73 ± 0.66	3.92 ± 0.68	3.93 ± 0.82	3.86 ± 0.10	Group	0.94
	PWS	27	3.85 ± 0.90	4.01 ± 0.68	3.77 ± 0.86	3.88 ± 0.10	Time	0.21
	PLA	27	3.93 ± 0.64	3.92 ± 0.61	3.63 ± 0.84	3.83 ± 0.10	G x T	0.35
	Mean ± SE		3.84 ± 0.08	3.95 ± 0.07	3.78 ± 0.09			
I am optimistic about my future performance	PWS+S	26	4.21 ± 0.75	4.30 ± 0.67	3.88 ± 0.99	4.13 ± 0.10	Group	0.25
	PWS	27	4.50 ± 0.63	4.42 ± 0.63	4.05 ± 0.66	4.32 ± 0.10	Time	< 0.001
	PLA	27	4.39 ± 0.49	4.16 ± 0.66	3.71 ± 0.95	4.09 ± 0.10	G x T	0.51
	Mean ± SE		4.37 ± 0.07	4.30 ± 0.07	3.88 ± 0.09 *^			
I feel vigorous and energetic	PWS+S	26	3.21 ± 0.80	3.00 ± 0.93 [b]	3.38 ± 0.85	3.20 ± 0.12	Group	0.26
	PWS	27	3.46 ± 0.94	3.46 ± 0.84 [a]	3.55 ± 0.73	3.49 ± 0.12	Time	0.17
	PLA	27	3.56 ± 0.71	3.24 ± 0.75	3.21 ± 0.93	3.34 ± 0.12	G x T	0.17
	Mean ± SE		3.41 ± 0.09	3.23 ± 0.09	3.38 ± 0.09			
My appetite is great	PWS+S	26	4.05 ± 0.92	3.96 ± 1.03	3.73 ± 1.11	3.91 ± 0.13 [c]	Group	0.13
	PWS	27	4.03 ± 0.81 [c]	4.03 ± 0.88	4.03 ± 0.79	4.04 ± 0.13	Time	0.11
	PLA	27	4.48 ± 0.64 [b]	4.25 ± 0.81	4.17 ± 0.79	4.30 ± 0.13 [a]	G x T	0.62
	Mean ± SE		4.19 ± 0.09	4.09 ± 0.10	3.97 ± 0.10			
I have little muscle soreness	PWS+S	26	3.52 ± 1.17	3.52 ± 1.17	3.73 ± 1.11	3.59 ± 0.14	Group	0.66
	PWS	27	3.56 ± 1.15	3.67 ± 1.11	3.52 ± 1.00	3.58 ± 0.13	Time	0.92
	PLA	27	3.84 ± 0.73	3.84 ± 0.99	3.58 ± 1.04	3.74 ± 0.13	G x T	0.68
	Mean ± SE		3.64 ± 0.11	3.67 ± 0.12	3.61 ± 0.11			

Values are means ± standard deviations. Six questions were analyzed by MANOVA. MANOVA analysis revealed overall Wilks' Lambda group ($p=0.27$), time ($p<0.001$), and group x time ($p=0.66$). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. [a] denotes a significant difference from PWS+S. [b] denotes a significant difference from PWS. [c] denotes a significant difference from PLA. * represents $p<0.05$ difference from baseline. ^ represents $p<0.05$ difference from wk 4.

Color test among groups over 8-wk following supplementation and there will be significant differences of RTP-VAS among groups over 8-wk following supplementation.

Exercise Performance

All strength testing outcomes are presented in Table 18. For bench press 1RM we observed significant increases in strength at week 4 for the PWS (8.17 kg; 95% CI 1.88, 14.47) and PWS+S (6.95 kg; 95% CI 0.62, 13.28), but not for the PLA (5.45 kg, 95% CI -0.82, 11.73). By week 8 all groups demonstrated a significant increase in 1RM-BP: PLA (7.18 kg, 95% CI 1.01, 13.36), PWS (14.36, 95% CI 8.13, 20.59) and PWS+S (13.84 kg, 95% CI 7.64, 20.04). No between groups differences were noted at week 4 or week 8 (Fig. 11a).

A similar pattern for change in 1RM-LP strength improvement at week 4 was observed within the PWS (61.84 kg, 95% CI 24.96, 98.72) and PWS+S (44.89 kg, 95% CI 8.31, 81.47) groups, but not for PLA (36.50, 95% CI, -0.21, 73.2). Similarly, by week 8, all groups increased their 1RM-LP as follows: PLA (43.28 kg, 95% CI 4.16, 82.41), PWS (79.23 kg, 95% CI 39.12, 118.54), and PWS+S (89.54 kg, 95% CI 50.55, 128.53). No between groups differences were noted at week 4 or week 8 (Fig. 11b). The results from the bench and leg press test analysis provides supporting evidence which rejected the null hypothesis of hypothesis 17 and 18 which stated that there will be significant differences of upper body strength with bench press test among groups over 8-wk following supplementation and there will be significant differences of lower body strength with leg press test among groups over 8-wk following supplementation.

Table 18. Study 2 - Strength Performance Characteristics

Variable	Group	N	Time (wk)			Mean ± SE		p-level
			0	4	8			
Bench Press (kg)	PWS+S	26	102.0 ± 16.3	106.2 ± 16.7	108.6 ± 17.1	105.6 ± 4.0	Group	0.56
	PWS	27	96.7 ± 23.1	99.2 ± 21.3	102.7 ± 21.7	99.5 ± 3.9	Time	< 0.001
	PLA	27	100.6 ± 20.8	102.9 ± 24.7	104.0 ± 23.3	102.5 ± 3.9	G x T	0.33
	Mean ± SE		99.8 ± 2.2	102.8 ± 2.3 *	105.1 ± 2.3 *^			
Leg Press (kg)	PWS+S	26	454.2 ± 79.4	474.4 ± 92.1	494.9 ± 100.9	474.5 ± 21.2	Group	0.67
	PWS	27	436.6 ± 96.6	466.0 ± 101.9	474.0 ± 96.3	458.9 ± 20.8	Time	< 0.001
	PLA	27	472.8 ± 149.8	490.3 ± 134.2	491.1 ± 131.0	484.7 ± 20.8	G x T	0.28
	Mean ± SE		454.5 ± 12.6	476.9 ± 12.4 *	486.7 ± 12.3 *^			

Values are means ± standard deviations. Strength (Bench and Leg Press) were analyzed by MANOVA. MANOVA analysis revealed overall Wilks' Lambda group (p=0.58), time (p<0.001), and group x time (p=0.29). Greenhouse-Geisser time and group x time interaction p-levels are reported with univariate group p-levels. [a] denotes a significant difference from PWS+S. [b] denotes a significant difference from PWS. [c] denotes a significant difference from PLA. * represents p<0.05 difference from baseline. ^ represents p<0.05 difference from wk 4.

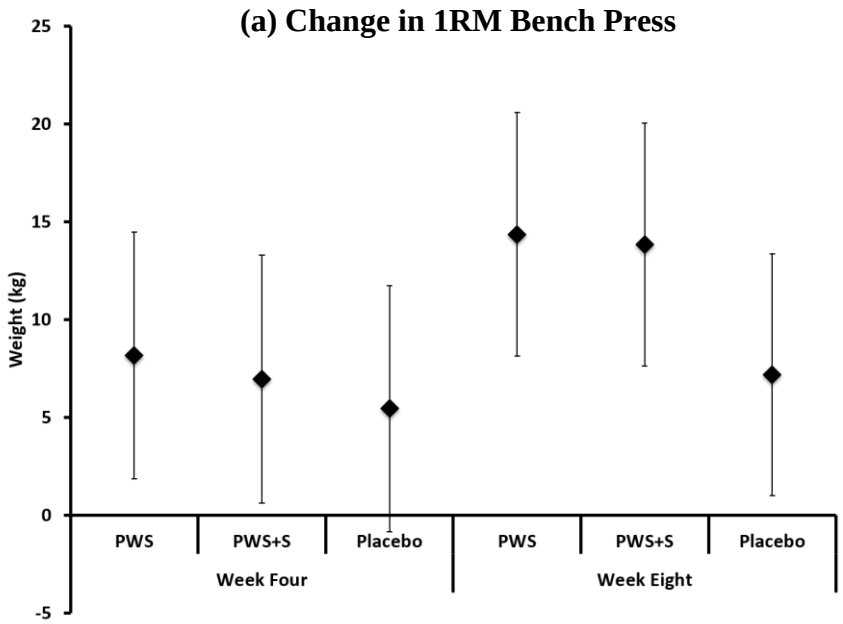

Figure 11a. Study 2 – Change in 1RM Bench Press. Data are mean change and 95% CI.

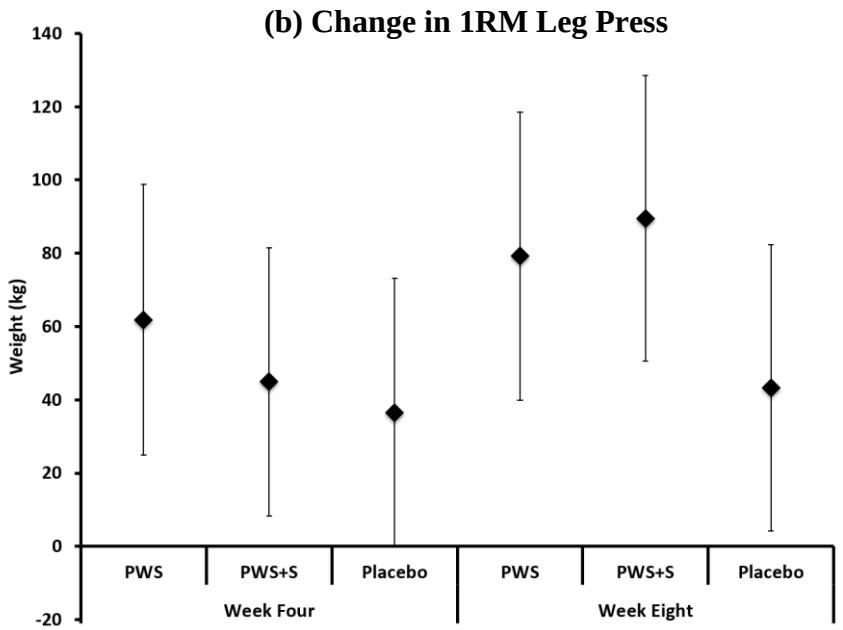

Figure 11b. Study 2 – Change in 1RM Leg Press. Data are mean change and 95% CI.

Lastly, as it pertains to performance, we did observe a significant increase in WAT peak power for PWS+S and PLA at week 4; yet no significant differences were otherwise noted at week 8 or other WAT parameters (Table 19). The results from the WAT analysis provides supporting evidence which rejected the null hypothesis of hypothesis 19 which stated that there will be significant differences of anaerobic capacity with WAT among groups over 8 weeks following supplementation.

Hematologic Characteristics

While we did observe several significant changes in hematological characteristics, these changes were random and occurred within all treatment groups, inclusive of the PLA (Table 20). Significant changes in blood chemistry are presented as follows. We observed significant time changes in BUN at week 8 for the PWS+S ($p < 0.01$) and PWS groups ($p < 0.001$), but no significant changes for the PLA group ($p = 0.21$); however, no between group significance was otherwise noted. Creatinine was significantly decreased at week four for the PLA ($p < 0.01$), PWS ($p < 0.01$) and PWS+S ($p = 0.01$) groups at week 4 and PLA group at week 8 ($p < 0.01$). There was no significant difference of creatinine between groups ($p = 0.27$), and post-hoc analyses showed no significant difference of PWS ($p = 0.24$) but significant difference of PWS+S ($p = 0.25$) vs. PLA. The BUN/Creatinine ratio was significantly elevated at week 4 in the PLA, PWS and PWS+S groups at week 4 and week 8 (all, $p < 0.001$). There was no significant difference for BUN:CRE ($p = 0.20$) between groups.

Significant changes in glucose were noted at week 4 for the PLA ($p < 0.01$), PWS ($p = 0.91$) and PWS+S ($p = 0.78$) groups and again at week 8 for the PLA ($p < 0.01$), PWS

Table 19. Study 2 - Wingate Anaerobic Capacity

Variable	Group	N	Time (wk)			Mean ± SE		p-level
			0	4	8			
Peak Power (Watt)	PWS+S	26	1445 ± 324	1605 ± 444	1544 ± 403	1532 ± 68	Group	0.91
	PWS	27	1502 ± 384	1602 ± 354	1603 ± 466	1569 ± 67	Time	< 0.01
	PLA	27	1472 ± 485	1630 ± 475	1507 ± 385	1536 ± 67	G x T	0.83
	Mean ± SE		1473 ± 45	1612 ± 47 *	1551 ± 46			
Mean Power (Watt)	PWS+S	26	630 ± 114	656 ± 134	612 ± 145	633 ± 20	Group	0.95
	PWS	27	630 ± 72	643 ± 66	638 ± 69	637 ± 19	Time	0.23
	PLA	27	647 ± 146	640 ± 133	636 ± 127	641 ± 19	G x T	0.40
	Mean ± SE		636 ± 12	646 ± 12	629 ± 13			
Peak Power (Watt/kg)	PWS+S	26	18.3 ± 3.6	20.2 ± 5.3	19.4 ± 4.8	19.3 ± 0.7	Group	0.90
	PWS	27	18.6 ± 4.7	19.8 ± 4.6	19.8 ± 5.8	19.4 ± 0.7	Time	0.01
	PLA	27	18.3 ± 5.6	20.2 ± 5.4	18.4 ± 4.0	19.0 ± 0.7	G x T	0.80
	Mean ± SE		18.4 ± 0.5	20.1 ± 0.5 *	19.2 ± 0.5			
Mean Power (Watt/kg)	PWS+S	26	8.0 ± 1.1	8.2 ± 1.4	8.0 ± 1.2	8.1 ± 0.2	Group	0.73
	PWS	27	7.8 ± 1.2	8.0 ± 1.1	7.9 ± 1.0	7.9 ± 0.2	Time	0.18
	PLA	27	8.0 ± 1.1	7.9 ± 1.0	7.7 ± 0.9	7.8 ± 0.2	G x T	0.33
	Mean ± SE		7.9 ± 0.1	8.0 ± 0.1	7.9 ± 0.1			
Total Work (Joule)	PWS+S	26	18923 ± 3431	19680 ± 4025	19213 ± 3110	19272 ± 629	Group	0.98
	PWS	27	18927 ± 2166	19319 ± 2003	19156 ± 2095	19134 ± 618	Time	0.17
	PLA	27	19394 ± 4377	19217 ± 4019	19109 ± 3826	19240 ± 618	G x T	0.29
	Mean ± SE		19081 ± 385	19405 ± 388	19159 ± 345			

Values are means ± standard deviations. Anaerobic performance (Peak Power, Mean Power, and Total Work) was analyzed by MANOVA. MANOVA analysis revealed overall Wilks' Lambda group (p=0.88), time (p=0.02), and group x time (p=0.85). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. * represents p<0.05 difference from baseline.

($p = 0.01$) and PWS+S ($p = 0.05$) groups. Finally, our assessment of hematology examining changes from "normal" to exceeding clinical limits showed no between group differences and, similar to individual markers of hepatorenal and muscle enzyme function, the changes associated with normal clinical status were randomly distributed amongst all treatment groups (Table 21). The results from the blood chemistry analysis provides supporting evidence which accepted the null hypothesis 20 through 23 which stated that there will be no significant differences of blood metabolic markers; cholesterol, HDL-C, LDL-C, glucose, LDH and CK, CRE and BUN, ALP, ALT, and AST, among groups over 8-wk following supplementation.

Table 20. Study 2 - Hematological Characteristics

Variable	Group	N	Time (wk) 0	4	8	Mean ± SE		p-level
ALP	PWS+S	26	76.4 ± 22.8	68.1 ± 17.3	74.4 ± 19.6	73.0 ± 3.5	Group	0.24
(U/L)	PWS	27	87.2 ± 24.7	72.3 ± 19.0	78.4 ± 24.9	79.3 ± 3.4	Time	< 0.001
	PLA	27	76.8 ± 15.1	66.4 ± 12.9	71.2 ± 13.4	71.5 ± 3.4	G x T	0.24
	Mean ± SE		80.1 ± 2.3	68.9 ± 1.8 *	74.7 ± 2.2 *^			
ALT	PWS+S	26	26.6 ± 23.0	28.6 ± 20.8	26.0 ± 12.2	27.1 ± 2.6	Group	0.74
(U/L)	PWS	27	25.1 ± 10.1	26.9 ± 16.0	30.9 ± 17.9	27.6 ± 2.5	Time	0.73
	PLA	27	25.3 ± 10.0	25.0 ± 22.4	24.6 ± 13.0	25.0 ± 2.5	G x T	0.55
	Mean ± SE		25.7 ± 1.7	26.8 ± 2.2	27.2 ± 1.6			
AST	PWS+S	26	28.4 ± 15.1	28.4 ± 10.3	27.3 ± 8.5	28.0 ± 2.1	Group	0.47
(U/L)	PWS	27	28.8 ± 9.7	31.6 ± 26.1	33.1 ± 19.7	31.1 ± 2.0	Time	0.96
	PLA	27	29.1 ± 11.0	27.6 ± 17.7	27.1 ± 9.8	27.9 ± 2.0	G x T	0.75
	Mean ± SE		28.7 ± 1.3	29.2 ± 2.1	29.1 ± 1.5			
BUN	PWS+S	26	13.6 ± 4.9	12.6 ± 6.6	16.5 ± 5.0	14.5 ± 0.7	Group	0.62
(mg/dl)	PWS	27	12.0 ± 4.0 [c]	12.3 ± 5.8	16.1 ± 5.3	13.5 ± 0.7	Time	< 0.001
	PLA	27	14.9 ± 4.9 [b]	12.3 ± 6.7	16.2 ± 6.8	14.2 ± 0.7	G x T	0.54
	Mean ± SE		13.5 ± 0.5	12.4 ± 0.7	16.3 ± 0.6 *^			
Creatinine	PWS+S	26	1.13 ± 0.51	0.82 ± 0.43	1.05 ± 0.28 [c]	1.01 ± 0.04	Group	0.27
(mg/dl)	PWS	27	1.08 ± 0.41	0.75 ± 0.38	0.95 ± 0.31	0.93 ± 0.04	Time	< 0.001
	PLA	27	1.15 ± 0.44	0.72 ± 0.47	0.85 ± 0.36 [a]	0.90 ± 0.04	G x T	0.78
	Mean ± SE		1.1 ± 0.0	0.7 ± 0.0 *	0.9 ± 0.0 *^			
BUN:Creatinine	PWS+S	26	15.3 ± 10.9	20.9 ± 15.1	17.9 ± 12.6	18.0 ± 1.8	Group	0.20
	PWS	27	12.7 ± 6.8	22.7 ± 17.3	20.4 ± 15.0	18.6 ± 1.8	Time	0.002
	PLA	27	15.7 ± 9.6	24.7 ± 19.1	26.5 ± 23.4	22.3 ± 1.8	G x T	0.62
	Mean ± SE		14.6 ± 1.0	22.8 ± 1.9	21.6 ± 1.9 *			
Glucose	PWS+S	26	90.7 ± 8.2	90.1 ± 5.0 [c]	95.4 ± 7.9	92.1 ± 1.2	Group	0.90
(mg/dl)	PWS	27	89.4 ± 7.1	89.7 ± 8.9 [c]	95.1 ± 9.2	91.4 ± 1.2	Time	< 0.001
	PLA	27	86.6 ± 7.0	93.7 ± 12.8 [a,b]	93.8 ± 13.0	91.3 ± 1.2	G x T	0.09
	Mean ± SE		88.9 ± 0.8	91.2 ± 1.0	94.8 ± 1.1 *^			

Values are means ± standard deviations. All variables were analyzed by MANOVA. MANOVA analysis revealed overall Wilks' Lambda group (p=0.47), time (p<0.001), and group x time (p=0.67). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. [a] denotes a significant difference from PWS+S. [b] denotes a significant difference from PWS. [c] denotes a significant difference from PLA. * represents p<0.05 difference from baseline. ^ represents p<0.05 difference from wk 4.

Table 20. Continued

Variable	Group	N	Time (wk) 0	Time (wk) 4	Time (wk) 8	Mean ± SE		p-level
Cholesterol	PWS+S	26	174.5 ± 42.8	171.9 ± 40.9	165.9 ± 36.1	170.8 ± 6.5	Group	0.34
(mg/dl)	PWS	27	164.1 ± 31.3	158.1 ± 26.0	163.3 ± 32.8	161.8 ± 6.4	Time	0.16
	PLA	27	159.5 ± 31.6	156.9 ± 34.3	156.1 ± 41.7	157.5 ± 6.4	G x T	0.43
	Mean ± SE		166.1 ± 3.9	162.3 ± 3.8	161.7 ± 4.1			
HDL-C	PWS+S	26	53.5 ± 16.2	52.7 ± 12.7	49.6 ± 15.0	51.9 ± 2.6	Group	0.99
(mg/dl)	PWS	27	52.7 ± 14.7	50.0 ± 13.9	52.6 ± 14.7	51.8 ± 2.5	Time	0.10
	PLA	27	53.4 ± 12.6	51.8 ± 13.1	51.3 ± 15.0	52.2 ± 2.5	G x T	0.24
	Mean ± SE		53.2 ± 1.6	51.5 ± 1.4	51.2 ± 1.6			
CHL:HDL	PWS+S	26	3.9 ± 3.7	3.5 ± 1.8	4.1 ± 4.4	3.8 ± 0.3	Group	0.38
	PWS	27	3.2 ± 0.9	3.3 ± 0.8	3.2 ± 0.7	3.2 ± 0.3	Time	0.35
	PLA	27	3.1 ± 0.7	3.1 ± 0.8	3.1 ± 0.7	3.1 ± 0.3	G x T	0.21
	Mean ± SE		3.4 ± 0.3	3.3 ± 0.1	3.5 ± 0.2			
LDL-C	PWS+S	26	121.0 ± 35.2	119.2 ± 42.3	116.2 ± 42.2	118.8 ± 6.4	Group	0.32
(mg/dl)	PWS	27	111.4 ± 29.8	108.0 ± 23.4	110.6 ± 27.9	110.0 ± 6.3	Time	0.43
	PLA	27	106.0 ± 29.6	105.0 ± 31.4	104.7 ± 35.1	105.2 ± 6.3	G x T	0.81
	Mean ± SE		112.8 ± 3.9	110.7 ± 3.7	110.5 ± 3.9			
Triglyceride	PWS+S	26	81.6 ± 36.6	73.9 ± 22.9 [b]	80.4 ± 41.9	78.6 ± 5.2	Group	0.11
(mg/dl)	PWS	27	92.8 ± 43.3	98.5 ± 37.8 [a]	86.5 ± 37.6	92.6 ± 5.1	Time	0.89
	PLA	27	74.2 ± 33.8	81.9 ± 31.5	83.6 ± 30.7	79.9 ± 5.1	G x T	0.31
	Mean ± SE		82.9 ± 4.2	84.8 ± 3.5	83.5 ± 4.1			
CK	PWS+S	26	504.6 ± 1075.5	382.6 ± 375.4	349.8 ± 316.2	412.3 ± 83.0	Group	0.34
(U/L)	PWS	27	395.6 ± 391.1	504.4 ± 1301.4	443.8 ± 607.8	447.9 ± 81.4	Time	0.79
	PLA	27	337.2 ± 255.7	263.1 ± 214.4	256.9 ± 242.7	285.7 ± 81.4	G x T	0.85
	Mean ± SE		412.4 ± 74.9	383.4 ± 88.9	350.2 ± 47.0			
LDH	PWS+S	26	158.8 ± 32.1	152.5 ± 24.3	160.4 ± 23.6	157.2 ± 4.9	Group	0.90
(U/L)	PWS	27	157.7 ± 24.2	160.7 ± 37.6	162.8 ± 24.3	160.4 ± 4.8	Time	0.18
	PLA	27	164.2 ± 36.2	153.5 ± 33.9	159.4 ± 29.5	159.0 ± 4.8	G x T	0.43
	Mean ± SE		160.2 ± 3.4	155.5 ± 3.6	160.9 ± 2.9			

Values are means ± standard deviations. All variables were analyzed by MANOVA. MANOVA analysis revealed overall Wilks' Lambda group ($p=0.47$), time ($p<0.001$), and group x time ($p=0.67$). Greenhouse-Geisser time and group x time (G x T) interaction p-levels are reported with univariate group p-levels. [a] denotes a significant difference from PWS+S. [b] denotes a significant difference from PWS. [c] denotes a significant difference from PLA. * represents $p<0.05$ difference from baseline. ^ represents $p<0.05$ difference from wk 4.

Table 21. Study 2 - Prevalence of Blood Chemistry Changes Exceeding Normal Clinical Bounds

			Placebo	PWS+S	PWS	Significance
Lipids & Glucose	**Cholesterol**	No Change	23	25	24	0.42
		Normal Baseline, Exceed at 4-wk	2	1	0	
		Normal Baseline, Exceed at 8-wk	0	0	1	
		Normal 4-wk, Exceed 8-wk	2	0	2	
	HDL-C	No Change	25	26	21	0.10
		Normal Baseline, Exceed at 4-wk	1	0	1	
		Normal Baseline, Exceed at 8-wk	1	0	1	
		Normal 4-wk, Exceed 8-wk	0	0	4	
	LDL-C	No Change	15	17	15	0.49
		Normal Baseline, Exceed at 4-wk	11	9	9	
		Normal Baseline, Exceed at 8-wk	1	0	1	
		Normal 4-wk, Exceed 8-wk	0	0	2	
	Triglycerides	No Change	27	25	27	0.35
		Normal Baseline, Exceed at 4-wk	0	1	0	
		Normal Baseline, Exceed at 8-wk	0	0	0	
		Normal 4-wk, Exceed 8-wk	0	1	0	
	Glucose	No Change	21	24	23	0.49
		Normal Baseline, Exceed at 4-wk	2	0	0	
		Normal Baseline, Exceed at 8-wk	1	0	1	
		Normal 4-wk, Exceed 8-wk	3	2	3	
Muscle	**LDH**	No Change	20	18	18	0.74
		Normal Baseline, Exceed at 4-wk	1	0	2	
		Normal Baseline, Exceed at 8-wk	2	3	1	
		Normal 4-wk, Exceed 8-wk	4	5	6	
	Creatine Kinase	No Change	19	21	22	0.42
		Normal Baseline, Exceed at 4-wk	1	0	0	
		Normal Baseline, Exceed at 8-wk	0	2	1	
		Normal 4-wk, Exceed 8-wk	7	3	4	

Data are frequency of occurrence. Significance is by chi-square analysis.

Table 21. Continued

			Placebo	PWS+S	PWS	Significance
Kidney	**Creatinine**	No Change	23	23	25	0.72
		Normal Baseline, Exceed at 4-wk	2	1	0	
		Normal Baseline, Exceed at 8-wk	0	0	0	
		Normal 4-wk, Exceed 8-wk	2	2	2	
	BUN	No Change	17	19	18	0.41
		Normal Baseline, Exceed at 4-wk	3	1	2	
		Normal Baseline, Exceed at 8-wk	0	2	0	
		Normal 4-wk, Exceed 8-wk	7	4	7	
Liver	**ALP**	No Change	25	25	23	0.28
		Normal Baseline, Exceed at 4-wk	1	0	0	
		Normal Baseline, Exceed at 8-wk	0	0	0	
		Normal 4-wk, Exceed 8-wk	1	1	4	
	ALT	No Change	24	22	22	0.72
		Normal Baseline, Exceed at 4-wk	1	0	0	
		Normal Baseline, Exceed at 8-wk	1	1	2	
		Normal 4-wk, Exceed 8-wk	1	3	3	
	AST	No Change	23	21	21	0.84
		Normal Baseline, Exceed at 4-wk	1	1	1	
		Normal Baseline, Exceed at 8-wk	0	2	1	
		Normal 4-wk, Exceed 8-wk	3	2	4	

Data are frequency of occurrence. Significance is by chi-square analysis.

CHAPTER V

DISCUSSION AND CONCLUSIONS

Study 1

The primary aim of Study 1 was to examine the acute effects of ingesting a dietary pre-workout supplement with and without synephrine. Safety was assessed via hematologic, and blood pressure and heart rate changes, and readiness was examined via the Stroop Test and Readiness to Perform questionnaires. When examined acutely, participants ingesting PWS and PWS+S demonstrated a consistent pattern regarding improving readiness to perform and cognitive function regardless of the presence of synephrine. While we did observe minor, yet significant changes in some indices of hepatorenal and metabolic function, it should be noted that the values remained within normal, clinical and expected ranges, demonstrated an irregular pattern, and were observed changes within all treatment groups, inclusive of PLA. Lastly, we did not observe any significant changes for exercise performance. Thus, we conclude that acute ingestion of the PWS examined in this study is safe and efficacious regarding readiness to performance and that the inclusion of synephrine to the PWS is unnecessary to achieve additional treatment effects.

Numerous studies have evaluated the effects of ingesting a PWS containing a variety of nutrients on readiness to perform, cognitive function, and exercise performance. Unfortunately, it is difficult to compare our results to those of others given the difference in formulations used in respective studies. For example, Little et al. [178] showed that the acute administration of combining creatine and α-ketoglutarate had a greater impact on

upper body muscle endurance and peak power output during repeated Wingate test than creatine alone. Similar studies have reported a greater time to exhaustion at 70% of VO_2 max, as well as a higher level of perceived focus, energy and less fatigue with PWS ingestion versus a placebo [10]. For cognitive function, the acute ingestion of herbal supplement, containing guarana, *Ginkgo biloba*, and elderberry, showed better performance of "vigilance", "focus ability", and "effectiveness at work" at 30 min and 120 min of following lunch with no adverse hemodynamic consequences [179].

Zak and colleagues [15] examined the acute effects of a multi-ingredient arginine-based supplement, including 3 g of arginine, 0.3 g of grape seed extract, and 0.3 g of polyethylene glycerol, on neuromuscular, ventilator, and metabolic fatigue thresholds during cycle ergometer and found the onset of neuromuscular fatigue delay and the ventilator threshold (VT) improvement in untrained individuals. Further, Gonzalez et al. [54] observed that an acute ingestion of a pre-workout supplement containing caffeine, β-alanine, and creatine significantly increased the number of repetitions performed at 80% of 1RM, as well as peak and mean power of bench press.

Despite the varied formulations used in the aforementioned studies, the primary difference in our current investigation is the inclusion or exclusion of synephrine. As such, few studies have been performed using synephrine in combination with other ingredients. Haller and associates [180] reported that the acute ingestion of caffeine (304 mg) and synephrine (21 mg) had no significant adverse effects although they noted a modest, yet normal increase in diastolic blood pressure accompanying supplementation. Ratamess et al. [181] investigated the effects of ingesting either synephrine alone (100 mg) or in

combination with caffeine (100 mg) for 3-d on resistance training performance indices. The researchers reported that both treatment groups increased their number of lifting repetitions and lifting volume vs. control and placebo groups. Further, mean power and lifting velocity was also reported to be higher for the caffeine and synephrine group. Consistent with our study, these authors reported no adverse effects in response to supplementation [181]. Finally, Gutierrez-Hellin [182] examined the acute effects of consumption of synephrine (3 mg/kg) on sprint performance in experienced sprinters undertaking series of performance sprint and power related exercise testing. Ingestion of synephrine did not increase exercise performance, nor did it cause any side effects. Similar to our findings, Stohs et al. [172] reported no significant changes in heart rate or blood pressure or self-reported symptomology accompanying the ingestion of 50 mg of synephrine with 100 to 1,000 mg of heparidin and 600 mg of naringin.

In the present study, we observed a significant increase in a VAS determined feelings of "*optimism about future performance*" and "*vigorous and energetic feeling*" using both treatments (i.e. with and without synephrine). Additionally, we found evidence that ingestion of the PWS prior to exercise increased some measures of cognitive function. However, the addition of synephrine to the PWS supplements used in our study did not provide additive benefit to perceptions about readiness to perform or feelings of energy/vigor. Our results are in agreement with Hoffman et al. [183] who observed a significantly greater feeling of energy and focus compared to placebo after ingesting a supplement containing various herbal and amino acids and are contrary to Gonzales et al. [54] who reported no significant difference in VAS determined feeling of energy when

ingesting a supplement containing caffeine, creatine, β-alanine. The improved perception about readiness to perform and cognitive function observed, however, did not translate into significant improvements in muscular endurance or anaerobic capacity. Thus, while perceptions about readiness to perform and cognitive function may have been improved, this did not result in greater exercise performance.

A strength of Study 1 is that we performed a well-controlled clinical trial examining the comprehensive acute effects of ingesting a PWS with and without synephrine on indices of readiness, cognition, and exercise performance in the same individuals. Additionally, we conducted a detailed analysis of markers of safety and observed no significant alterations in respective blood pressure, heart rate and hematologic variables with either supplement form. A weakness of this study is that these results can only be attributed to the PWS studied and additional research is needed to determine the acute and chronic effects of ingesting other PWS on cognitive and exercise performance capacity as well as safety.

Study 2

The primary aim of Study 2 was to examine the chronic effect of PWS with and without synephrine for 8 weeks on training adaptation in resistance trained athletes. In this study, we focused on the long term exercise performance and cognitive function while also monitoring for safety. Accordingly, we did observe several significant improvements in strength and cognitive function performance favoring a PWS and that synephrine was not necessary to achieve these effects. Accompanying these findings was a lack of significant effects on blood pressure, heart rate or hematological changes over the 8-wk

for either treatment group. While a few indices of hepatorenal and metabolic function did show significant changes, inclusive of the PLA group, it should be noted that the values did not demonstrate a consistent pattern for any treatment group. This becomes readily apparent when examining Table 21, which reports on those individuals exceeding clinical limits at specific time points during the study period. As it pertains to body composition, we did not observe any significant changes in DXA determined aspects of anthropometry. Our results suggest that a PWS with and without synephrine is safe and efficacious regarding readiness and exercise performance and that the inclusion of synephrine is unnecessary to achieve the observed treatment effects, though it does appear that synephrine does offer better overall improvements in cognition when taken acutely.

While much attention has been given to single nutrition ergogenic aids, fewer studies have examined the effects of multi-ingredient supplements and their potential effects on physical performance. Fewer still have examined additional factors such as readiness to perform and cognitive function. A comparison between our study and earlier work is difficult as each pre-workout formula can differ with regard to ingredients. While earlier studies generally focused on variations of creatine, amino acids, β-alanine and various admixtures of ingredients, newer investigations have begun to examine nitrates as an adjunct to re-ingestion formula. For example, in 2009, Hoffman et al. [183] examined the effects of ingesting a pre-exercise energy drink containing β-alanine and caffeine as the primary ingredients of a multi-ingredient formula and found no significant differences in measurements of anaerobic power despite participants reporting higher subjective levels feeling energized and focused. These latter effects were complimented by greater number

of targets struck during a Makato Test. In similar study ingesting a multi-ingredient supplement containing 400 mg of phosphatidylserine and 100 mg of caffeine, 2-wk supplement intake showed to attenuate post-exercise mood scores and perception of fatigue, while not to affect cognitive function or reaction time [184].

Similar reports by others have observed a greater time to exhaustion at 70% of VO_2 max, as well as a higher level of perceived focus, energy and less fatigue versus a placebo [10]. In the same year, Smith et al. [11] reported on the effects of ingesting a pre-workout supplement containing caffeine, creatine, and various amino acids during 3-wk of high-intensity interval training, which resulted in an increase in VO_2 max, overall training volume, lean body mass increase and a decrease in percent body fat versus those taking a placebo. Shortly after this time period, nitrates, primarily from beetroot juice, were introduced as a viable pre-workout ingestion strategy and in 2013, Lowery et al. [13] demonstrated that a multi-ingredient supplement containing branched chain amino acids, creatine monohydrate, β-alanine, quercetin, coenzymated B-vitamins, alanyl-glutamine and nitrate from pomegranate and beetroot extracts increased upper body strength, muscle hypertrophy and muscle thickness. Our study therefore adds to the literature of using nitrate, in combination with other ingredients, as a pre-workout strategy. Overall, our study showed no adverse effects of blood pressure, heart rate or hematological characteristics typically associated with hepatorenal or muscle enzymes alterations. This is important as recent criticisms suggest that the evidence supporting multi-ingredient formulas do improve performance, but are matched by inclusive and incomplete reports

regarding safety and side effects. Thus, of our report strengthens and enhances the literature via its thorough reporting of possible side effects.

A major strength of Study 2 is that it elaborates on the findings of Study 1 lasting just a few hours. The results of the combined studies demonstrate no adverse hematological or blood pressure, heart rate effects following the acute or chronic ingestion of a pre-workout supplement. Further PWS and PWS+S increased readiness and cognitive function acutely and chronically based on the results from Stroop testing. While we did observe significant strength increase, we did not observe change in body composition.

Another strength is that we compared a multi-ingredient formula with and without synephrine, which also has had concerns raised regarding safety. Similar to our findings, Kaats et al. [167] reported no significant changes in hemodynamic or hematological safety indices accompanying 49 mg/d or 98 mg/d of synephrine ingestion for 60-d. These findings are cumulatively supported by a review of Stohs et al. [185]. An interesting finding of Study 2 is that synephrine does not appear to add to the overall effectiveness of the formula we investigated when taken chronically.

Summary and Conclusion

In summary, results from Study 1 assessing the acute effects of PWS supplementation with and without synephrine suggest that acute ingestion of PWS can improve cognitive function and perceptions of readiness to perform but has no impact on bench press, leg press, or anaerobic sprint capacity. Adding synephrine to the PWS did not significantly affect resting energy expenditure or provide additive effects to cognitive or exercise performance. Results also suggest that acute ingestion of the PWS's did not

significantly affect hematological or blood pressure, heart rate variables following the ingestion of a pre-workout supplement for a short-term; 60 min to 120 min.

During Study 1, we observed a significant increase in a RTP assessment demonstrating increased feeling of "optimism about future performance" and "vigorous and energetic feeling" using both treatments (i.e. with and without synephrine). Our results, however, did not persist through the chronic ingestion for 8-wk of our trial. Interestingly, our use of the Stroop Word-Color initially demonstrated quite a different effect whereby; both treatment groups exhibited a significant increase in respective measurement indices during the acute study, while the effect observed during chronic study of our trial demonstrated a nearly two-fold higher affect for the PWS vs. PLA or PWS+S conditions.

Thus, we suggest that the pre-workout supplement examined here is safe when taken both acutely and chronically. Also, our findings support PWS beneficial effects to physical and cognitive performance while it is equally effective whether or not it contains synephrine.

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APPENDIX A. STUDY 1 CONSENT FORM

TEXAS A&M UNIVERSITY HUMAN SUBJECTS PROTECTION PROGRAM
CONSENT FORM

Project Title: **Pharmacokinetic, Thermogenic, Hemodynamic, Ergogenic Assessment of a Pre-Workout Dietary Supplement**

You are invited to take part in a research study being conducted by Dr. Richard Kreider, a researcher from Texas A&M University and funded by Woodbolt International. The information in this form is provided to help you decide whether or not to take part. If you decide to take part in the study, you will be asked to sign this consent form. If you decide you do not want to participate, there will be no penalty to you, and you will not lose any benefits you normally would have.

Why Is This Study Being Done?
The purpose of this study is to examine the acute effects of a pre-workout dietary supplement on energy metabolism, cardiovascular hemodynamics, blood metabolites and mental focus.

Why Am I Being Asked To Be In This Study?
You are being asked to be in this study because you are an apparently healthy and recreationally active man or woman between the ages of 18 and 40. You will need to have at least 6 months immediate prior history of resistance training on the bench press and leg press or squat. You will not be allowed to participate if; you have a history of treatment for metabolic disease (i.e., diabetes), hypertension, hypotension, thyroid disease, arrhythmias and/or cardiovascular disease; you are currently using any prescription medications; you have an intolerance to caffeine and/or other natural stimulants; you are pregnant or a lactating female or plan to become pregnant within the next month; you have a history of smoking; or you drink excessively (12 drinks per week or more). If you do not qualify for this study we will keep your contact information (phone number and/or e-mail) and contact you at a later date for potential entry into a similar study.

How Many People Will Be Asked To Be In This Study?
Approximately 20 people (participants) will be invited to participate in this study locally.

What Are the Alternatives to being in this study?
The alternative to being in the study is not to participate.

What Will I Be Asked To Do In This Study?
You will be asked to not exercise for 48 hours nor eat or drink calorie containing foods or drinks 12 hours before each testing session/visit. In addition you will be asked to refrain from ingesting caffeine and over the counter medication with known stimulant use for 48 hours. Your participation in this study will last up to approximately three weeks and include four visits (visit 1 ~ 1 hour/visits 2, 3 & 4 ~ 3 hours/visit). These visits are detailed below and in Table 1.

Visit 1 (week 0) – Familiarization (T1)
This visit will last about one hour. During this visit the details of the study will be explained, human subject consent forms and radiation consent forms will be signed, personal and medical history information will be completed and you will have a general physical that will include measurement of fasting blood to determine if you can participate in the study. You will donate approximately 5 ml (about 1 teaspoon) of fasting blood from a vein in your arm according to standard procedures. Next you will have you height measured, your weight measured and your

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total body composition measured. You will then be asked to perform a 1 repetition maximum test on the bench press and leg press and be familiarized to the 30 second Wingate test.

Visit 2, 3 & 4 (week 1, 2 & 3) – (T2, T3 and T4)
These visits will last about three hours. You will first donate approximately 20 ml (about 4 teaspoons) of fasting blood from a vein in your arm according to standard procedures. Next cognitive function tests, readiness to perform visual analogue scale (VAS), resting blood pressure (BP), resting heart rate (HR), resting electrocardiographs (ECG) and resting energy expenditure (REE) will be measured for 10 minutes. You will then be randomized and counterbalanced to ingest your assigned pre-workout dietary supplement. You will then have REE and ECGs continuously measured for 30 minutes with BP and HR measured every 10 minutes after which a second 20 ml blood sample will be taken. You will next be asked to perform a second cognitive function test, rate readiness to perform on a VAS, warm up and then perform 3 sets of 10 repetitions at 70% of 1 repetition maximum on the bench press and leg press with 2 minutes recovery between sets and 5 minutes recovery from exercise modes. You will then be asked to complete as many repetitions as possible during the final set. Following a 5 minute recovery, you will be asked to perform a Wingate 30 second anaerobic capacity test on a cycle ergometer. You will then be asked to perform a third cognitive function test, rate readiness to perform and asked to donate a third and final 20 ml blood sample. You will be asked to repeat these procedures two additional times using an alternate supplement following a one week washout after each testing session. In the event of an emergency during an exercise test proper emergency response protocols (calling 9-911 for serious injury or a medical emergency, calling Biosafety/EHS for cleanup assistance or spill team response, calling UPD for incidents in public areas, retrieving AED located in the lab, performing CPR or other First Aid techniques, etc.) will be followed by the Exercise & Sport Nutrition Laboratory (ESNL) staff depending on the severity of the emergency.

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Table 1 - Protocol Overview

Familiarization(T1)	T2	T3	T4
Phone Screening	Fasting Blood Sample	Fasting Blood Sample	Fasting Blood Sample
Familiarization	Cognitive Function Test	Cognitive Function Test	Cognitive Function Test
Physical Exam	Readiness to Perform VAS	Readiness to Perform VAS	Readiness to Perform VAS
Body Weight	12-Lead Resting ECG	12-Lead Resting ECG	12-Lead Resting ECG
DEXA Body Composition 1RM Determination	REE (10-min) and resting HR & BP	REE (10-min) and resting HR & BP	REE (10-min) and resting HR & BP
Anaerobic Sprint Practice Test Practice	Ingest supplement in randomized and counterbalanced manner	Ingest supplement in randomized and counterbalanced manner	Ingest supplement in randomized and counterbalanced manner
Schedule Testing Refrain from exercise, caffeine and use of over-the-counter stimulants for 48 hours prior to each testing session	REE for 30-min with HR & BP determined every 10-min and arrhythmias documented (if any)	REE for 30-min with HR & BP determined every 10-min and arrhythmias documented (if any)	REE for 30-min with HR & BP determined every 10-min and arrhythmias documented (if any)
	Blood Sample	Blood Sample	Blood Sample
	Cognitive Function Test	Cognitive Function Test	Cognitive Function Test
	Readiness to Perform VAS	Readiness to Perform VAS	Readiness to Perform VAS
	Bench Press Warm-Up	Bench Press Warm-Up	Bench Press Warm-Up
	Bench Press (3 sets of 10 reps @ 70% or 1RM with 2-min rest recovery between sets. Total reps to failure on last set)	Bench Press (3 sets of 10 reps @ 70% or 1RM with 2-min rest recovery between sets. Total reps to failure on last set)	Bench Press (3 sets of 10 reps @ 70% or 1RM with 2-min rest recovery between sets. Total reps to failure on last set)
	5-min rest	5-min rest	5-min rest
	Leg Press Warm-up	Leg Press Warm-up	Leg Press Warm-up
	Leg Press (3 sets of 10 reps @ 70% or 1RM with 2-min rest recovery between sets. Total reps to failure on last set)	Leg Press (3 sets of 10 reps @ 70% or 1RM with 2-min rest recovery between sets. Total reps to failure on last set)	Leg Press (3 sets of 10 reps @ 70% or 1RM with 2-min rest recovery between sets. Total reps to failure on last set)
	5-min rest	5-min rest	5-min rest
	Wingate AC Test	Wingate AC Test	Wingate AC Test
	Cognitive Function Test	Cognitive Function Test	Cognitive Function Test
	Readiness to Perform VAS	Readiness to Perform VAS	Readiness to Perform VAS
	Blood Sample	Blood Sample	Blood Sample
	1-week washout	1-week washout	

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You may be removed from the study by the investigator for these reasons:

- You do not show up for your scheduled testing sessions/visits and the investigators are unable to contact you to reschedule.
- You do not follow your assigned supplemental protocol
- You do not follow you assigned exercise protocol

Are There Any Risks To Me?

The things that you will be doing are greater than risks that you would come across in everyday life. Although the researchers have tried to avoid risks, you may feel that some questions/procedures that are asked of you will be stressful or upsetting. You do not have to answer anything you do not want to. You will be exposed to a low level of radiation during the body composition test, which is similar to the amount of natural background radiation you would receive in one month while living in College Station Texas. The use of the body composition scanner has been shown to be a safe method of measuring body composition and is approved by the FDA. You will donate approximately 5 ml (about 1 teaspoon) of fasting blood during the initial familiarization/screening visit and then approximately 20 ml (about 4 teaspoons) of blood three times at each of the three testing sessions throughout the study using standard procedures. These procedures may cause a small amount of pain when the needle is inserted into the vein as well as some bleeding and bruising. You may also experience some dizziness and/or faint if you are unaccustomed to having blood drawn. The exercise tests that will be performed may cause symptoms of fatigue, shortness of breath and/or muscular fatigue/discomfort. The exercise tests may also cause short-term muscle soreness and moderate fatigue for several days following the tests. You may also experience muscle strains/pulls during the exercise testing and/or training program. However, exercise session will be conducted by trained personnel and monitored to ensure you follow appropriate exercise guidelines.

Are There Any Benefits To Me?

The direct benefit to you by being in this study is to know more about your health and fitness status from the tests to be performed. However, even if no individual benefit is obtained, you will be paid for your participation.

Will There Be Any Costs To Me?

Aside from your time, there are no costs for taking part in the study.

Will I Have To Pay Anything If I Get Hurt In This Study?

If you suffer any injury as a result of taking part in this research study, please understand that nothing has been arranged to provide free treatment of the injury or any other type of payment. However, all needed facilities, emergency treatment and professional services will be available to you just as they are to the community in general. You should report any injury to Dr. Richard Kreider at 979-845-1333. You will not give up any of your legal rights by signing this consent form.

Side effects (injury) can happen in any research study. These effects may not be your fault or the fault of the researcher involved. Known side effects have been described in the "Are there any risks to me?" section of this consent form. However, side effects that are not currently known may happen and require care. You do not give up any of your legal rights by signing this form.

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Will I Be Paid To Be In This Study?
You will receive a total of $100 ($25 for each visit) in one check at the end of the study. Payment will occur after finishing all four sessions and after all study materials (questionnaires, etc.) have been turned in to the study staff. You will be paid on a prorated basis if you are unable to complete the entire study.

Will Information From This Study Be Kept Private?
The records of this study will be kept private. No identifiers linking you to this study will be included in any sort of report that might be published. Research records will be stored securely and only Exercise & Sport Nutrition Laboratory staff will have access to the records.

Information about you will be stored in locked file cabinets in a locked file room in an ID card swipe access controlled laboratory. Computer files will be protected with a password. This consent form will be filed securely in an official area.

People who have access to your information include the Principal Investigator and research study personnel. Representatives of regulatory agencies such as the Office of Human Research Protections (OHRP) and entities such as the Texas A&M University Human Subject's Protection Program may access your records to make sure the study is being run correctly and that information is collected properly.

The agency that is funding this study (Woodbolt International) and the institution(s) where study procedures are being performed (Texas A&M University) may also see your information. However, any information that is sent to them will be coded with a number so that they cannot tell who you are. Representatives from these entities can see information that has your name on it if they come to the study site to view records. If there are any reports about this study, your name will not be in them.

Information about you and related to this study will be kept confidential to the extent permitted or required by law.

Who may I Contact for More Information?
You may contact the Principal Investigator, Richard Kreider, PhD, to tell him about a concern or complaint about this research at 979-845-1333 or rkreider@hlkn.tamu.edu. You may also contact the Protocol Director/Laboratory Research Associate, Chris Rasmussen, at 979-458-1741 or crasmussen@hlkn.tamu.edu.

For questions about your rights as a research participant; or if you have questions, complaints, or concerns about the research, you may call the Texas A&M University Human Subject's Protection Program office at (979) 458-4067 or irb@tamu.edu.

What if I Change My Mind About Participating?
This research is voluntary and you have the choice whether or not to be in this research study. You may decide to not begin or to stop participating at any time. If you choose not to be in this study or stop being in the study, there will be no effect on your student status, medical care, employment, evaluation, relationship with Texas A&M University, etc. Any new information

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discovered about the research will be provided to you. This information could affect your willingness to continue your participation.

STATEMENT OF CONSENT

I agree to be in this study and know that I am not giving up any legal rights by signing this form. The procedures, risks, and benefits have been explained to me, and my questions have been answered. I know that new information about this research study will be provided to me as it becomes available and that the researcher will tell me if I must be removed from the study. I can ask more questions if I want. A copy of this entire consent form will be given to me.

Participant's Signature	Date
Printed Name	Date

INVESTIGATOR'S AFFIDAVIT:

Either I have or my agent has carefully explained to the participant the nature of the above project. I hereby certify that to the best of my knowledge the person who signed this consent form was informed of the nature, demands, benefits, and risks involved in his/her participation.

Signature of Presenter	Date
Printed Name	Date

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APPENDIX B. STUDY 2 CONSENT FORM

TEXAS A&M UNIVERSITY HUMAN SUBJECTS PROTECTION PROGRAM
CONSENT FORM

Project Title: **Effects of Pre-Workout Dietary Supplement on Training Adaptations in Resistance Trained Athletes**

You are invited to take part in a research study being conducted by Dr. Richard Kreider, a researcher from Texas A&M University and funded by Woodbolt International. The information in this form is provided to help you decide whether or not to take part. If you decide to take part in the study, you will be asked to sign this consent form. If you decide you do not want to participate, there will be no penalty to you, and you will not lose any benefits you normally would have.

Why Is This Study Being Done?
The purpose of this study is to examine the effects of a pre-workout dietary supplement during resistance training on training adaptations.

Why Am I Being Asked To Be In This Study?
You are being asked to be in this study because you are an apparently healthy and recreationally active male between the ages of 18 and 40. You will need to have at least 6 months immediate prior history of resistance training on the bench press and leg press or squat. You will not be allowed to participate if; you have a history of treatment for metabolic disease (i.e., diabetes), hypertension, hypotension, thyroid disease, arrhythmias and/or cardiovascular disease; you are currently using any prescription medications; you have an intolerance to caffeine and/or other natural stimulants; you have a history of smoking; or you drink excessively (12 drinks per week or more). If you do not qualify for this study we will keep your contact information (phone number and/or e-mail) and contact you at a later date for potential entry into a similar study with your permission.

How Many People Will Be Asked To Be In This Study?
Approximately 72 people (participants) will be invited to participate in this study locally.

What Are the Alternatives to being in this study?
The alternative to being in the study is not to participate.

What Will I Be Asked To Do In This Study?
You will be asked to not exercise for 48 hours nor eat or drink calorie containing foods or drinks 12 hours before each testing session/visit. In addition you will be asked to refrain from ingesting caffeine and over the counter medication with known stimulant use for 48 hours. Your participation in this study will last approximately nine weeks and include six visits (visit 1 ~ 1 hour/visit 2 & 5 ~ 30 min/visit 3, 4 & 6 ~ 3 hours/visit). You will provide two muscle biopsies during this study. These visits are detailed below and in Table 1.

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Table 1 – Protocol Overview

Familiarization(T1)	T2 (week 1)		T3 (week 4)	T4 (week 8)	
Visit 1	Biopsy ~ 1 day prior Visit 2	Performance testing Visit 3	Performance testing Visit 4	Biopsy ~ 1 day prior Visit 5	Performance testing Visit 6
Phone Screening Familiarization Physical Exam Body Weight 1 Repetition Maximum Practice Familiarization Anaerobic Sprint Test Practice Schedule Testing Refrain from exercise, caffeine and use of over-the-counter stimulants for 48-hours prior to each testing session	Muscle Biopsy	4-Day Food Record 48 hour non-exercise 12 hour fast Fasting Blood Sample Body Weight Body Water Body Composition Readiness to Perform Scale & Cognitive Function Test Bench Press Warm-Up Bench Press 1 Repetition Maximum with 2-min rest recovery between sets. 5-min rest Leg Press Warm-up Leg Press 1 Repetition Maximum with 2-min rest recovery between sets. 5-min rest Wingate Anaerobic Sprint Test Administration of supplements Begin training	4-Day Food Record 48 hour non-exercise 12 hour fast Fasting Blood Sample Body Weight Body Water Body Composition Readiness to Perform Scale & Cognitive Function Test Bench Press Warm-Up Bench Press 1 Repetition Maximum with 2-min rest recovery between sets. 5-min rest Leg Press Warm-up Leg Press 1 Repetition Maximum with 2-min rest recovery between sets. 5-min rest Wingate Anaerobic Sprint Test	Muscle Biopsy	4-Day Food Record 48 hour non-exercise 12 hour fast Fasting Blood Sample Body Weight Body Water Body Composition Readiness to Perform Scale & Cognitive Function Test Bench Press Warm-Up Bench Press 1 Repetition Maximum with 2-min rest recovery between sets. 5-min rest Leg Press Warm-up Leg Press 1 Repetition Maximum with 2-min rest recovery between sets. 5-min rest Wingate Anaerobic Sprint Test

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Visit 1 – Familiarization (T1)
This visit will last about one hour. During this visit the details of the study will be explained, human subject consent forms will be signed, personal and medical history information will be completed and you will have a general physical that will include measurement of fasting blood to determine if you can participate in the study. You will donate approximately 5 ml (about 1 teaspoon) of fasting blood from a vein in your arm according to standard procedures. Next you will have your height and weight measured. You will then be familiarized to the bench press, leg press and anaerobic sprint test. Next you will be given a food log and asked to record all calorie containing food and drinks for a total of four days (including one weekend day) prior to your second visit.

Visit 2 & 5 (week 1 & 8) – (T2 & T4 – Biopsy)
These visits will last about 30 minutes and will take place approximately one day prior to the T2 and T4 performance testing sessions. Biopsies will be obtained from the middle portion of the thigh muscle between the knee and the upper leg using the Bergstrom biopsy (muscle tissue sample) technique. All biopsies will be taken by trained and experienced personnel in the Human Countermeasures Laboratory or the Exercise & Sport Nutrition Laboratory at Texas A&M University. The muscle biopsy procedure involves the following: First, you will be asked to lie down or assume a comfortable reclining position on an exam table. The biopsy technician will identify the point where the biopsy will be obtained. The area will be shaved clean of any leg hair, washed with a sterilizing soap, cleaned with rubbing alcohol, and further cleansed by swabbing the area with a fluid sterilizing soap and then draped with sterile padding. A small area of the thigh approximately 2 cm in diameter will be anesthetized with a 1.0 mL injection of 2% Xylocaine/Lidocaine (a numbing agent). Once the local anesthesia has taken effect (approximately 2-5 minutes) each biopsy procedure will take approximately 15-20 minutes. A scalpel point will be used to produce the initial biopsy site by making an incision approximately 1 cm. in length through the skin, subcutaneous fat, and fascia. Due to the localized effects of the anesthetic, you will feel little to no pain during this process. The biopsy needle will be advanced into the incision approximately ½ inch. During this part of the procedure you may feel pressure to the thigh area. The biopsy procedure will obtain approximately 100 – 200 micro-grams of muscle tissue (smaller than a pencil eraser). Once the muscle sample has been obtained, direct pressure will be immediately applied. The site will be subsequently closed, and the wound dressed with a pressure bandage. Due to the small incision site, only minimal bleeding is expected. Afterwards, written instructions for post-biopsy care will be reviewed and issued. You will be instructed to leave the bandages on for 24 hours (unless unexpected bleeding or pain occurs) and asked to report back to the lab within 24 hours to have the old bandages removed, the incision inspected and new bandages applied. These suggestions will minimize pain and possible bleeding of the area. If needed, you may take non-prescription analgesic medication such as Tylenol to relieve pain. Soreness of the area may occur for about 24 hours post-biopsy.

Visit 3, 4 & 6 (week 1, 4 & 8) – (T2, T3 & T4 - Performance Testing)
These visits will last about three hours. You will first donate approximately 20 ml (about 4 teaspoons) of fasting blood from a vein in your arm according to standard procedures. Next resting blood pressure, resting heart rate, body water, body composition, a reediness to perform scale and a cognitive function test will be completed. You will then be asked to perform a warm-up and one repetition maximum test on the bench press and leg press with a two minute

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recovery between attempts and a five minute recovery between exercises. Next you will be asked to warm up on a cycle ergometer and perform a 30 second sprint test on a bicycle.

You will then be matched according to age, body mass, fat free mass and training history and be assigned your pre-workout dietary supplement. Figure 1 shows the ingredient list for the pre-workout dietary supplement (per 2 servings or 12 g). You will be asked to ingest two scoops of the supplement mixed with water approximately 15 to 30 minutes prior to exercise training sessions. On non-training days, you will be asked to ingest two scoops of the supplement with breakfast. You will be asked to follow a standardized resistance training program and record the amount of weight lifted and repetitions performed on training logs. You will also be asked to record supplement intake and complete weekly medical side effects questionnaires. After four and eight weeks of training/supplementation, you will be asked to repeat baseline testing with the exception that muscle biopsies will only be taken at one and eight weeks of training/supplementation.

Figure 1. Ingredient list for the pre-workout dietary supplement (per 2 servings or 12 g)

Dietary Ingredients:

- Beta Alanine, 3000 mg
- Creatine Nitrate, 2000 mg
- Arginine AKG, 2000 mg
- Ascorbic Acid (Vitamin C), 250 mg
- N-Acetyl L-Tyrosine (proprietary amount)
- Caffeine Anhydrous, 135 mg
- *Mucuna pruriens* extract (15% L-Dopa) (proprietary amount)
- Niacin (as Niacinamide), 30 mg
- Bitter Orange peel extract (*Citrus aurantium*)(30% Synephrine)(proprietary amount)
- Vitamin B6 (as Pyridoxal 5-Phosphate), 1.0 mg
- Folate (as Folic Acid), 500 mcg
- Vitamin B12 (as Methylcobalamin), 70 mcg

Other Ingredients:

- Citric Acid (proprietary amount)
- Natural and Artificial Flavors (proprietary amount)
- Silicon Dioxide (proprietary amount)
- Calcium Silicate (contains 22 mg calcium)
- Sucralose (proprietary amount)
- FD&C Red Lake #40 (proprietary amount)
- Acesulfame Potassium (Ace K) (proprietary amount)

You may be removed from the study by the investigator for these reasons:

- You do not show up for your scheduled testing sessions/visits and the investigators are unable to contact you to reschedule.
- You do not follow your assigned supplemental protocol
- You do not follow your assigned exercise protocol

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Are There Any Risks To Me?
The things that you will be doing are greater than risks that you would come across in everyday life. Although the researchers have tried to avoid risks, you may feel that some questions/procedures that are asked of you will be stressful or upsetting. You do not have to answer anything you do not want to. You will be exposed to a low level of radiation three times during the body composition exam, which is similar to the amount of natural background radiation you would receive in one month while living in College Station Texas. In addition, a very low level of electrical current will be passed through your body using a bioelectrical impedance analyzer. This analyzer is commercially available and has been used in the health care/fitness industry as a means to assess body composition and body water for over 20 years. The use of the body composition scanner and bioelectrical impedance analyzer have been shown to be safe methods of assessing body composition and total body water and are approved by the FDA. You will donate approximately 5 ml (about 1 teaspoon) of fasting blood during the initial familiarization/screening visit and then approximately 20 ml (about 4 teaspoons) of blood three additional times throughout the duration of the study using standard procedures. These procedures may cause a small amount of pain when the needle is inserted into the vein as well as some bleeding and bruising. You may also experience some dizziness and/or faint if you are unaccustomed to having blood drawn. Similar risks as well as minimal bleeding, soreness and bruising may be involved with the two muscle biopsy procedures. There is a slight risk of contracting an infection. However, only a trained phlebotomist will be performing blood sampling using previously approved sterile procedures. The biopsy procedure may also carry a risk of soreness (100%), infection (<1%), and permanent numbness (<<1%). Additional risks include discomfort, bleeding and possible scarring at the biopsy site. The exercise tests that will be performed may cause symptoms of fatigue, shortness of breath and/or muscular fatigue/discomfort. The exercise tests may cause short-term muscle soreness and moderate fatigue for several days following the tests. You may also experience muscle strains/pulls during the exercise testing and/or training program. However, exercise session will be conducted by trained personnel and monitored to ensure you follow appropriate exercise guidelines.

Are There Any Benefits To Me?
The direct benefit to you by participating in this study is to know more about your health and fitness status from the tests to be performed. However, even if no individual benefit is obtained, you will be paid for your participation.

Will There Be Any Costs To Me?
Aside from your time, there are no costs for taking part in the study.

Will I Have To Pay Anything If I Get Hurt In This Study?
If you suffer any injury as a result of taking part in this research study, please understand that nothing has been arranged to provide free treatment of the injury or any other type of payment. However, all needed facilities, emergency treatment and professional services will be available to you just as they are to the community in general. You should report any injury to Dr. Richard Kreider at 979-845-1333. You will not give up any of your legal rights by signing this consent form.

Side effects (injury) can happen in any research study. These effects may not be your fault or the fault of the researcher involved. Known side effects have been described in the "Are there any

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risks to me?" section of this consent form. However, side effects that are not currently known may happen and require care. You do not give up any of your legal rights by signing this form.

Will I Be Paid To Be In This Study?
You will receive a total of $200 ($25 for each visit plus $50 for each biopsy visit) in one check at the end of the study. Payment will occur after completing all six sessions and after all study materials (food logs, training logs, questionnaires, etc.) have been turned in to the study staff. You will be paid on a prorated basis if you are unable to complete the entire study.

Will Information From This Study Be Kept Private?
The records of this study will be kept private. No identifiers linking you to this study will be included in any sort of report that might be published. Research records will be stored securely and only Exercise & Sport Nutrition Laboratory staff will have access to the records.

Information about you will be stored in locked file cabinets in a locked file room in an ID card swipe access controlled laboratory. Computer files will be protected with a password. This consent form will be filed securely in an official area.

People who have access to your information include the Principal Investigator and research study personnel. Representatives of regulatory agencies such as the Office of Human Research Protections (OHRP) and entities such as the Texas A&M University Human Subject's Protection Program may access your records to make sure the study is being run correctly and that information is collected properly.

The agency that is funding this study (Woodbolt International) and the institution where study procedures are being performed (Texas A&M University) may also see your information. However, any information that is sent to them will be coded with a number so that they cannot tell who you are. Representatives from these entities can see information that has your name on it if they come to the study site to view records. If there are any reports about this study, your name will not be in them.

Information about you and related to this study will be kept confidential to the extent permitted or required by law.

Who may I Contact for More Information?
You may contact the Principal Investigator, Richard Kreider, PhD, to tell him about a concern or complaint about this research at 979-845-1333 or rkreider@hlkn.tamu.edu. You may also contact the Protocol Director/Laboratory Research Associate, Chris Rasmussen, at 979-458-1741 or crasmussen@hlkn.tamu.edu.

For questions about your rights as a research participant; or if you have questions, complaints, or concerns about the research, you may call the Texas A&M University Human Subject's Protection Program office at (979) 458-4067 or irb@tamu.edu.

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What if I Change My Mind About Participating?
This research is voluntary and you have the choice whether or not to be in this research study. You may decide to not begin or to stop participating at any time. If you choose not to be in this study or stop being in the study, there will be no effect on your student status, medical care, employment, evaluation, relationship with Texas A&M University, etc. Any new information discovered about the research will be provided to you. This information could affect your willingness to continue your participation.

STATEMENT OF CONSENT
I agree to be in this study and know that I am not giving up any legal rights by signing this form. The procedures, risks, and benefits have been explained to me, and my questions have been answered. I know that new information about this research study will be provided to me as it becomes available and that the researcher will tell me if I must be removed from the study. I can ask more questions if I want. A copy of this entire consent form will be given to me.

_____ _____
Participant's Signature Date

_____ _____
Printed Name Date

INVESTIGATOR'S AFFIDAVIT:
Either I have or my agent has carefully explained to the participant the nature of the above project. I hereby certify that to the best of my knowledge the person who signed this consent form was informed of the nature, demands, benefits, and risks involved in his/her participation.

_____ _____
Signature of Presenter Date

_____ _____
Printed Name Date

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